

NOVEL GENE TARGETS AND LIGANDS THAT BIND THERETO  
FOR TREATMENT AND DIAGNOSIS OF COLON CARCINOMAS

RELATED APPLICATIONS

5 This application relates to U.S. Provisional Patent Application Serial No. 60/367,727 filed March 28, 2002 , U.S. Provisional Patent Application Serial No. 60/381,328 filed May 20, 2002, U.S. Provisional Patent Application Serial No. 60/386, 747 filed June 10, 2002, and U.S. Provisional Patent Application Serial No. 60/427,564 filed November 20, 2002, each of which are incorporated by reference in their entirety herein.

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FIELD OF THE INVENTION

The present invention relates the identification of gene targets for treatment and diagnosis of neoplastic diseases, such as colon or colorectal cancer, and other cancers wherein the subject genes are upregulated and the use thereof to express the corresponding  
15 antigen, and to produce ligands that specifically bind such antigen, e.g. monoclonal antibodies and small molecules.

DESCRIPTION OF RELATED ART

Colorectal cancers are among the most common cancers in men and women in the  
20 U.S. and are one of the leading causes of death. Other than surgical resection no other systemic or adjuvant therapy is available. Vogelstein and colleagues have described the sequence of genetic events that appear to be associated with the multistep process of colon cancer development in humans (Fearon and Vogelstein, 1990). An understanding of the molecular genetics of carcinogenesis, however, has not led to preventative or therapeutic  
25 measures. It can be expected that advances in molecular genetics will lead to better risk assessment and early diagnosis but colorectal cancers will remain a deadly disease for a majority of patients due to the lack of an adjuvant therapy.

Endogenous gastrins and exogenous gastrins (other than tetragastrin) seem to promote the growth of established colon cancers in mice (Singh, et al., 1986; Singh, et al., 1987; et al.,  
30 1984; Smith and Solomon, 1988; Singh, et al., 1990; Rehfeld and van Solinge, 1994) and promote carcinogen induced colon cancers in rats (Williamson et al., 1978; Karlin et al., 1985; Lamoste and Willems; 1988). Recent studies of Montag et al (1993) further support a possible co-carcinogenic role of gastrin in the initiation of tumors.

Many colon cancer cells express and secrete gastrin gene products (Dai et al., 1992;

Kochinan et al., 1992; Finley et al., 1993; Van Solinge et al., 1993; Xu et al., 1994; Singh et al., 1994a; Hoosein et al., 1988; Hoosein et al., 1990) and bind gastrin-like peptides (Singh et al., 1986; Singh et al., 1987; Weinstock and Baldwin, 1988; Watson and Steele, 1994; Upp et al., 1989; Singh et al., 1985). In previous reports gastrin antibodies were either reported to  
5 inhibit (Hoosein et al., 1988; Hoosein et al., 1990) the growth of colon cancer cell lines *in vitro*.

However other investigators have had inconclusive results with colon cancer cell lines. A number of studies testing the effects of gastrin on cell proliferation of cancer cells have been performed (Sirinek et al., 1985; Kusk et al., 1986; Watson et al., 1989). The  
10 results have varied widely. In one study, four different human cancer cell lines were tested for growth stimulation by pentagastrin and only one showed growth stimulation (Eggstein et al., 1991). Similarly in majority of the studies conducted to-date, mitogenic effects of gastrin have been demonstrated only on a very small percentage of colon cancer cell lines (Hoosein et al., 1988; Hoosein et al., 1990; Shrink et al., 1985; Kusk et al., 1986; Guo et al., 1990;  
15 Ishizuka et al., 1994).

Since only a small percentage of established human colon cancer cell lines demonstrated a growth response to exogenous gastrins, investigators in this field came to believe that gastrin probably did not play a significant role in the growth of colon cancers. The recent discovery that human colon cancer cell lines and primary human colon cancers  
20 express the gastrin gene has sparked a renewed interest in a possible autocrine role of gastrin-like peptides in colon cancers. However, significant skepticism remains in the field, to date, regarding the importance of gastrin gene expression to the continued growth and tumorigenicity of colon cancers.

Thus, to-date, no systemic or adjuvant therapies have been developed for colon  
25 cancers, based on the knowledge that a significant percentage of human colon cancers express the gastrin gene. In fact, no adjuvant or systemic therapy has been developed for colon cancers that is based on the knowledge of the expression of other growth factors such as TGF- $\alpha$  or IGF-II, since none of the growth factors demonstrate a significant growth effect on majority of the colon cancer cell lines in culture.

30 At the present time the only systemic treatment available for colon cancer is chemotherapy. However, chemotherapy has not proven to be very effective for the treatment of colon cancers for several reasons, in part because colon cancers express high levels of the

MDR gene (that codes for multi-drug resistance gene products). The MDR gene products actively transport the toxic substances out of the cell before the chemotherapeutic agents can damage the DNA machinery of the cell. These toxic substances harm the normal cell populations more than they harm the colon cancer cells for the above reasons.

5           There is no effective systemic treatment for treating colon cancers other than surgically removing the cancers. In the case of several other cancers, including breast cancers, the knowledge of growth promoting factors (such as EGF, estradiol, IGF-II) that appear to be expressed or effect the growth of the cancer cells, has been translated for treatment purposes. But in the case of colon cancers this knowledge has not been applied and  
10 therefore the treatment outcome for colon cancers remains bleak.

          Antisense RNA technology has been developed as an approach to inhibiting gene expression, including oncogene expression. An "antisense" RNA molecule is one which contains the complement of, and can therefore hybridize with, protein-encoding RNAs of the cell. It is believed that the hybridization of antisense RNA to its cellular RNA complement  
15 can prevent expression of the cellular RNA, perhaps by limiting its translatability. While various studies have involved the processing of RNA or direct introduction of antisense RNA oligonucleotides to cells for the inhibition of gene expression (Brown, et al., 1989; Wickstrom, et al., 1988; Smith, et al., 1986; Buvoli, et al., 1987), the more common means of cellular introduction of antisense RNAs has been through the construction of recombinant  
20 vectors that express antisense RNA once the vector is introduced into the cell.

          A principle application of antisense RNA technology has been in connection with attempts to affect the expression of specific genes. For example, Delauney, et al. have reported the use antisense transcripts to inhibit gene expression in transgenic plants (Delauney, et al., 1988). These authors report the down-regulation of chloramphenicol acetyl  
25 transferase activity in tobacco plants transformed with CAT sequences through the application of antisense technology.

          Antisense technology has also been applied in attempts to inhibit the expression of various oncogenes. For example, Kasid, et al., 1989, report the preparation of recombinant vector construct employing Craf-1 cDNA fragments in an antisense orientation, brought  
30 under the control of an adenovirus 2 late promoter. These authors report that the introduction of this recombinant construct into a human squamous carcinoma resulted in a greatly reduced tumorigenic potential relative to cells transfected faith control sense transfectants. Similarly,

Prochownik, et al., 1988, have reported the use of Cmiyc antisense constructs to accelerate differentiation and inhibit G.sub.1 progression in Friend Murine Erythroleukemia cells. In contrast, Khokha, et al., 1989, discloses the use of antisense RNAs to confer oncogenicity on 3T3 cells, through the use of antisense RNA to reduce murine tissue inhibitor or  
5 metalloproteinases levels.

Antisense methodology takes advantage of the fact that nucleic acids tend to pair with "complementary" sequences. By complementary, it is meant that polynucleotides are those which are capable of base-pairing according to the standard Watson-Crick complementary rules. That is, the larger purines base pair with the smaller pyrimidines to form combinations  
10 of guanine paired with cytosine (G:C) and adenine paired with either thymine (A:T) in the case of DNA, or adenine paired with uracil (A:U) in the case of RNA. Inclusion of less common bases such as inosine, 5-methylcytosine, 6-methyladenine, hypoxanthine and others in hybridizing sequences does not interfere with pairing.

Targeting double-stranded (ds) DNA with polynucleotides leads to triple-helix  
15 formation; targeting RNA leads to double-helix formation. Antisense polynucleotides, when introduced into a target cell, specifically bind to their target polynucleotide and interfere with transcription, RNA processing, transport, translation and/or stability. Antisense RNA constructs, or DNA encoding such antisense RNAs, can be employed to inhibit gene transcription or translation or both within a host cell, either *in vitro* or *in vivo*, such as within  
20 a host animal, including a human subject.

Throughout this application, the term "expression vector or construct" is meant to include any type of genetic construct containing a nucleic acid coding for a gene product in which part or all of the nucleic acid encoding sequence is capable of being transcribed. The transcript can be translated into a protein but it need not be. Thus, in certain embodiments,  
25 expression includes both transcription of a gene and translation of mRNA into a gene product. In other embodiments, expression only includes transcription of the nucleic acid encoding a gene of interest.

The nucleic acid encoding a gene product is under transcriptional control of a promoter. A "promoter" refers to a DNA sequence recognized by the synthetic machinery of  
30 the cell, or introduced synthetic machinery, required to initiate the specific transcription of a gene. The phrase "under transcriptional control" means that the promoter is in the correct location and orientation in relation to the nucleic acid to control RNA polymerase initiation



and expression of the gene.

The term promoter is used to refer to a group of transcriptional control modules that are clustered around the initiation site for RNA polymerase II. Much of the thinking about how promoters are organized derives from analyses of several viral promoters, including those for the HSV thymidine kinase (tk) and SV40 early transcription units. These studies, augmented by more recent work, have shown that promoters are composed of discrete functional modules, each consisting of approximately 7-20 base pairs of DNA, and containing one or more recognition sites for transcriptional activator or repressor proteins.

At least one module in each promoter functions to position the start site for RNA synthesis. The best known example of this is the TATA box, but in some promoters lacking a TATA box, such as the promoter for the mammalian terminal deoxynucleotidyl transferase gene and the promoter for the SV40 late genes, a discrete element overlying the start site itself helps to fix the place of initiation.

Additional promoter elements regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 base pairs upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the tk promoter, the spacing between promoter elements can be increased to 50 base pairs apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription.

A promoter is selected based on its capability to direct gene expression in the targeted cell. Thus, where a human cell is targeted, the nucleic acid coding region can be positioned adjacent to and under the control of a promoter that is capable of being expressed in a human cell. Generally speaking, such a promoter might include either a human or viral promoter.

In various instances, the human cytomegalovirus (CMV) immediate early gene promoter, the SV40 early promoter and the Rous sarcoma virus long terminal repeat can be used to obtain high-level expression of the gene of interest. The use of other viral or mammalian cellular or bacterial phage promoters which are well known in the art to achieve expression of a gene of interest is contemplated as well, provided that the levels of expression are sufficient for a given purpose.

By employing a promoter with well-known properties, the level and pattern of expression of the gene product following transfection can be optimized. Further, selection of a promoter that is regulated in response to specific physiologic signals can permit inducible expression of the gene product. Representative elements/promoters useful in accordance with the present invention include but are not limited to those listed below.

Enhancers were originally detected as genetic elements that increased transcription from a promoter located at a distant position on the same molecule of DNA. This ability to act over a large distance had little precedent in classic studies of prokaryotic transcriptional regulation. Subsequent work showed that regions of DNA with enhancer activity are organized much like promoters. That is, they are composed of many individual elements, each of which binds to one or more transcriptional proteins.

The basic distinction between enhancers and promoters is operational. An enhancer region as a whole must be able to stimulate transcription at a distance; this need not be true of a promoter region or its component elements. A promoter includes one or more elements that direct initiation of RNA synthesis at a particular site and in a particular orientation, whereas enhancers lack these specificities. Promoters and enhancers are often overlapping and contiguous, often seeming to have a very similar modular organization.

Viral promoters, cellular promoters/enhancers and inducible promoters/enhancers that could be used in combination with the nucleic acid encoding a gene of interest in an expression construct. Some examples of enhancers include Immunoglobulin Heavy Chain; Immunoglobulin Light Chain; T-Cell Receptor; HLA DQ a and DQ b b-Interferon; Interleukin-2; Interleukin-2 Receptor; Gibbon Ape Leukemia Virus; MHC Class II 5 or HLA-DRa; b-Actin; Muscle Creatine Kinase; Prealbumin (Transthyretin); Elastase I; Metallothionein; Collagenase, Albumin Gene;  $\alpha$ -Fetoprotein;  $\alpha$ -Globin;  $\beta$ -Globin; c-fos; c-HA-ras; Insulin Neural Cell Adhesion Molecule (NCAM);  $\alpha$ 1-Antitrypsin; H2B (TH2B) Histone; Mouse or Type I Collagen; Glucose-Regulated Proteins (GRP94 and GRP78); Rat Growth Hormone; Human Serum Amyloid A (SAA); Troponin I (TN I); Platelet-Derived Growth Factor; Duchenne Muscular Dystrophy; SV40 or CMV; Polyoma; Retroviruses; Papilloma Virus; Hepatitis B Virus; Human Immunodeficiency Virus. Inducers such as phorbol ester (TFA) heavy metals; glucocorticoids; poly (rl)X; poly(rc); Ela; H<sub>2</sub>O<sub>2</sub>; IL 1; Interferon, Newcastle Disease Virus; A23187; IL-6; Serum; SV40 Large T Antigen; FMA; thyroid Hormone; could be used. Additionally, any promoter/enhancer combination (as per

the Eukaryotic Promoter Data Base EPDB) could also be used to drive expression of the gene. Eukaryotic cells can support cytoplasmic transcription from certain bacterial promoters if the appropriate bacterial polymerase is provided, either as part of the delivery complex or as an additional genetic expression construct.

5 In certain instances, the expression construct can comprise a virus or engineered construct derived from a viral genome. The ability of certain viruses to enter cells via receptor-mediated endocytosis and to integrate into host cell genome and express viral genes stably and efficiently have made them attractive candidates for the transfer of foreign genes into mammalian cells (Ridgeway, 1988; Nicolas and Rubenstein, 1988; Baichwal et al., 1986; 10 Temin, 1986). The first viruses used as gene vectors were DNA viruses including the papoviruses (simian virus 40, bovine papilloma virus, and polyoma) (Ridgeway, 1988; Baichwal et al., 1986) and adenoviruses (Ridgeway, 1988; Baichwal et al., 1986). These have a relatively low capacity for foreign DNA sequences and have a restricted host spectrum. Furthermore, their oncogenic potential and cytopathic effects in permissive cells raise safety 15 concerns. They can accommodate only up to 8 kB of foreign genetic material but can be readily introduced in a variety of cell lines and laboratory animals (Nicolas and Rubenstein, 1988; Temin, 1986).

Where a cDNA insert is employed, a polyadenylation signal is typically inserted to effect proper polyadenylation of the gene transcript. Any suitable polyadenylation sequence 20 can be used. An expression cassette can also include a terminator sequence. These elements enhance message levels and minimize read through from the cassette into other sequences.

It is understood in the art that to bring a coding sequence under the control of a promoter, or operatively linking a sequence to a promoter, one positions the 5' end of the transcription initiation site of the transcriptional reading frame of the protein between about 25 1 and about 50 nucleotides "downstream" of (*i.e.*, 3' of) the chosen promoter. In addition, where eukaryotic expression is contemplated, an appropriate polyadenylation site (*e.g.*, 5'-AATAAA-3' (SEQ ID NO:66)) can be included if absent from the original cloned segment. Typically, the poly A addition site is placed about 30 to 2000 nucleotides "downstream" of the termination site of the protein at a position prior to transcription termination.

30 The above background references are part of the present invention insofar as they are applicable to the invention described herein. Hence there are no effective and specific ways of treating or diminishing the growth of colorectal cancer to date.

Therefore, there exists a significant need for the identification of novel gene targets for the treatment and diagnosis of colon or colorectal cancer, especially given the huge human toll caused by this disease annually.

#### SUMMARY OF THE INVENTION

It is an aspect of the invention to identify novel gene targets for treatment and the diagnosis of cancer, such as colon or colorectal cancer.

It is a specific aspect of the invention to develop novel therapies for treatment of cancer, such as colon cancer, involving the administration of anti-sense oligonucleotides corresponding to gene targets that are expressed by certain colon or colorectal cancers.

It is another specific aspect of the invention to provide the antigens expressed by genes that are expressed by malignant tissues, *e.g.*, colon or colorectal cancers.

It is another specific aspect of the invention to produce ligands that bind antigens expressed by certain cancers, such as colon or colorectal cancers. Representative ligands include monoclonal antibodies.

It is another specific aspect of the invention to provide novel therapeutic regimens for the treatment of cancer, for example colon cancer, that involve the administration of antigens expressed by certain colon or colorectal cancers, alone or in combination with adjuvants that elicit an antigen-specific cytotoxic T-cell lymphocyte response against cancer cells that express such antigen.

It is another aspect of the invention to provide novel therapeutic regimens for the treatment of cancer, such as colon or colorectal cancer, that involve the administration of ligands, for example, monoclonal antibodies that specifically bind novel antigens that are expressed by certain cancer tissues including colon cancer tissues.

It is another aspect of the invention to provide a novel method for diagnosis of cancer, for example colon or colorectal cancer, by using ligands, *e.g.*, monoclonal antibodies, that specifically bind to antigens that are expressed by cancers including certain colon or colorectal cancers, in order to detect whether a subject has or is at increased risk of developing colon or colorectal cancer.

It is another aspect of the invention to provide a novel method of detecting persons having, or at increased risk of developing certain types of cancers, including colon cancer by

use of labeled DNAs that hybridize to novel gene targets expressed by certain cancers, including colon cancers.

It is yet another aspect of the invention to provide diagnostic test kits for the detection of persons having or at increased risk of developing certain cancer, including colon cancer that comprise a ligand, *e.g.*, monoclonal antibody that specifically binds to an antigen  
 5 expressed by certain colon cancers, and a detectable label, *e.g.*, a radiolabel or fluorophore.

It is another aspect of the invention to provide diagnostic kits for detection of persons having or at risk of developing certain cancers, including colon cancer that comprise DNA primers or probes specific for novel gene targets expressed by colon cancers, and a detectable  
 10 label, *e.g.* radiolabel or fluorophore.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 summarizes expression data for the CICO1, CICO2 and CICO3, which were identified based on overexpression in colon cancer as described in Example 1.

Figures 2-5 depict gene expression profiles determined using the Gene Logic  
 15 datasuite as described in Example 2. The values along the y-axis represent expression intensities in Gene Logic units. Each blue circle represents an individual patient sample. The bar graph on the left of the figure depicts the percentage of each tissue type found to express the gene fragment. The total number of samples for each tissue type is as follows: colon  
 20 tumor, tumor % above 50, 31; colon tumors, 45; normal breast, 37; normal colon, 30; normal esophagus, 18, normal kidney, 28; normal liver, 21; normal lung, 35; normal lymph node 10; normal ovary, 25; normal pancreas, 20; normal prostate, 20; normal rectum, 22; normal stomach, 25. "Colon tumor, tumor % above 50" refers to tumor samples for which at least  
 25 50% of each sample comprises malignant tissue, as determined by a pathologist. This sample set is a subset of colon tumors, which comprises all colon tumor samples contained within the Gene Logic database.

Figure 2 depicts the gene expression profile of Candidate 1, which was determined using the Gene Logic datasuite for GENBANK Accession No. W91975 as described in Example 2. Candidate 1 is overexpressed in colon tumor tissue.

Figure 3 depicts the gene expression profile of Candidate 2, which was determined  
 30 using the Gene Logic datasuite for GENBANK Accession No. A1694242 as described in Example 2. Candidate 2 is overexpressed in colon tumor tissue.

Figure 4 contains the gene expression profile of Candidate 3, which was determined using the Gene Logic datasuite for GENBANK Accession No. AI680111 as described in Example 2. Candidate 3 is overexpressed in colon tumor tissue.

5 Figure 5 depicts the gene expression profile of Candidate 4, which was determined using the Gene Logic datasuite for GENBANK Accession No. AA813827 as described in Example 2. Candidate 4 is overexpressed in colon tumor tissue.

10 Figures 6A and 6B show PCR data of Candidate 3 expression (Figure 6A) and GAPDH expression (Figure 6B) in normal human tissues. Candidate 3 was screened against Human Multiple Tissue cDNA panels I & II (Clontech #K1420-1 & # K1421-1 ) according to the manufacturer's instructions. GAPDH was not tested against the prostate sample. The positive control for Candidate 3 was IMAGE 2324560, obtained from the American Tissue Type Collection (Manassas, Virginia). The cDNA samples present in each lane are as follows: lane 1, heart; lane 2, brain; lane 3, placenta; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; lane 8, pancreas; lane 9, spleen; lane 10, thymus; lane 11, prostate; lane 12, testis; lane 13, ovary; lane 14, small intestine; lane 15, colon; lane 16, peripheral blood leukocytes; lane 17, positive control; lane 18, negative control. Arrow denotes the anticipated size of the PCR product for candidate 3. The results shown in this figure indicate that candidate 3 is not expressed at detectable levels in any of the normal tissues tested.

20 Figures 7A and 7B show PCR data of Candidate 3 expression (Figure 7A) and GAPDH expression (Figure 7B) in colon tumor samples. The cDNA samples present in each lane are as follows: lane 1, grade 3 adenocarcinoma; lane 2, grade 2 adenocarcinoma; lane 3, grade 1 adenocarcinoma; lane 4, grade 2 adenocarcinoma; lane 5, colorectal cancer cell line HCT116; lane 6, positive control (IMAGE clone); lane 7, negative control. Arrow denotes the anticipated size of the PCR product for candidate 3. The results shown in this figure indicate that candidate 3 is expressed in at least 3 of 4 colon tumor samples in addition to colorectal tumor cell line HCT116.

Figure 8 depicts E-Northern expression data for Loc 56926, which is overexpressed in colon cancer, as described in Example 4.

30 Figures 9A and 9B are PCR panels showing expression of Loc56926 (Figure 9A) and GAPDH (Figure 9B) in malignant colon samples. The cDNA samples present in each lane are as follows: lane M, marker; lane 1, no template control; lane 2 colon cancer 8T; lane 3, colon cancer DT; lane 4, colon cancer FT; lane 5, colon cancer GT; lane 6, colon cancer HT;

lane 7, colon cancer IT; lane 8, colon cancer QT; lane 9, prostate cancer OT; lane 10, colon cancer RT; lane 11, colon cancer cell line HCT116; lane 12, positive control EST. The results from this figure demonstrate that Loc56926 expression is present in cDNA from three of eight tested colon cancer samples.

5           Figures 10A and 10B are PCR panels showing expression of Loc56926 (Figure 10A) and GAPDH (Figure 10B) in normal human tissues. Hybridization was performed using Human Multiple Tissue cDNA panel I (Clontech #K1420-1) according to the manufacturer's instructions. The cDNA samples present in each lane are as follows: lane M, marker; lane 1, no template control; lane 2, colon tumor 8T; lane 3, colon tumor HT; lane 4, colon tumor RT;  
10   lane 5, colon cancer cell line HCT116; lane 6, normal colon; lane 7, normal brain; lane 8, normal heart; lane 9, kidney; lane 10, normal liver; lane 11, normal lung; lane 12, skeletal muscle; lane 13, normal pancreas; lane 14, normal placenta lane 15; EST control. These results demonstrate that Loc56926 is present in colon tumors with light expression in the normal pancreas (note the increase in GAPDH in the pancreas lane compared to the colon  
15   tumor lanes) and not expressed at detectable levels the other tested normal human tissues.

          Figures 11A and 11B are PCR panels showing expression of Loc56926 (Figure 11A) and GAPDH (Figure 11B) in human tissues. Hybridization was performed using Human Multiple Tissue cDNA panel II (Clontech # K1421-1) according to the manufacturer's instructions. The cDNA samples present in each lane are as follows: lane M, marker; lane 1,  
20   no template control; lane 2, colon tumor 8T; lane 3, colon tumor HT; lane 4, colon tumor RT; lane 5, colon cancer cell line HCT116; lane 6, normal colon; lane 7, normal peripheral blood leukocytes; lane 8, small intestine; lane 9, normal ovary; lane 10, normal prostate; lane 11, normal spleen; lane 12, normal testis; lane 13, normal thymus; lane 14, EST control. These results demonstrate that Loc56926 is not expressed at detectable levels in these normal  
25   tissues.

          Figures 12A and 12B are PCR panels showing expression of Loc56926 (Figure 12A) and GAPDH (Figure 12B) in normal brain tissue samples. Hybridization was performed using Normal Neural System cDNA panel (Biochain, C8234503, C8234504, C8234505). The cDNA samples present in each lane are as follows: lane M, marker; lane 1, no template  
30   control; lane 2, cerebellum; lane 3, cerebral cortex; lane 4, medulla oblongata; lane 5, pons; lane 6, frontal lobe; lane 7, occipital lobe; lane 8, parietal lobe; lane 9, temporal lobe; lane 10,

placental neural system; lane 11, EST control. These results demonstrate that Lco56926 is not expressed at detectable levels in the normal brain.

Figure 13 depicts E-Northern expression data for the AW779536 gene, which is overexpressed in colon cancer, as described in Example 4.

5        Figure 14 depicts E-Northern expression data for the AL531683 gene, which is overexpressed in colon cancer, as described in Example 4.

Figure 15 depicts E-Northern expression data for the AI202201 gene, which is overexpressed in colon cancer, as described in Example 4.

10       Figure 16 depicts E-Northern expression data for the AL389942 gene, which is overexpressed in colon cancer, as described in Example 4.

Figure 17 depicts E-Northern expression results for the Ly6G6Dgene, also described in Example 5.

Figure 18 depicts E-Northern expression results for FLJ32334, also described in Example 6.

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#### DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the identification of genes which are to be specifically expressed and upregulated in certain cancers, including colon or colorectal tumors. This was determined using the Gene Logic (Gaithersburg, Maryland) datasuite or Celera (Rockville, Maryland) database and by screening malignant colon tumor tissues as described in detail herein.

In particular, the present invention involves the discovery that certain genes, the nucleic acid sequences and predicted coding sequences of which are identified herein are specifically expressed in certain malignant tissues including colon or colorectal tumor tissues.

25       The disclosed therapies involve the synthesis of oligonucleotides having sequences in the antisense orientation relative to the genes identified by the present inventors which are specifically expressed by malignant tissues, including colon or colorectal tumors. Suitable therapeutic antisense oligonucleotides typically vary in length from two to several hundred nucleotides in length, more typically about 50-70 nucleotides in length. These antisense  
30       oligonucleotides can be administered as naked DNAs or in protected forms, *e.g.*, encapsulated in liposomes. The use of liposomal or other protected forms may enhance *in vivo* stability and delivery to target sites, *i.e.*, colon tumor cells.



Also, the subject novel genes can be used to design novel ribozymes that target the cleavage of the corresponding mRNAs in colon and other tumor cells. Similarly, these ribozymes can be administered in free (naked) form or by the use of delivery systems that enhance stability and/or targeting, *e.g.*, liposomes. Ribozymal and antisense therapies used to target genes that are selectively expressed by cancer cells are well known in the art.

Also, the present invention embraces the administration of use of DNAs that hybridize to the novel gene targets identified herein, attached to therapeutic effector moieties, for example radiolabels, including metallic and halogen isotopes (*e.g.*, <sup>90</sup>yttrium, <sup>131</sup>iodine), cytotoxins, cytotoxic enzymes, in order to selectively target and kill cells that express these genes, *i.e.*, colon tumor cells.

Still further, the present invention encompasses non-nucleic acid based therapies, for example antigens encoded by the nucleic acids disclosed herein. It is anticipated that these antigens can be used as therapeutic or prophylactic anti-tumor vaccines. For example, antigens of the present invention can be administered with adjuvants that induce a cytotoxic T lymphocyte response. Representative adjuvants include those disclosed in U.S. Patent Nos. 5,709,860, 5,695,770, and 5,585,103, which promote CTL responses against prostate and papillomavirus related human colon cancer. The disclosures of U.S. Patent Nos. 5,709,860, 5,695,770, and 5,585,103 are incorporated by reference in their entirety.

The disclosed antigens can be administered in combination with an adjuvant to elicit a humoral immune response against such antigens, thereby delaying or preventing the development of cancers (*e.g.*, a colon cancer) associated with the overexpression of the antigens.

Embodiments of the invention comprise administration of one or more novel colon cancer antigens, for example in combination with an adjuvant. A representative adjuvant is PROVAX®, which comprises a microfluidized adjuvant containing Squalene, TWEEN® and PLURONIC®, in an amount sufficient to be therapeutically or prophylactically effective. *See* U.S. Patent Nos. 5,709,860, 5,695,770, and 5,585,103. A typical dosage of formulated antigen ranges from about 50 to about 20,000 mg/kg body weight, or from about 100 to about 5000 mg/kg body weight.

Alternatively, the subject tumor-associated antigens can be administered with other adjuvants, *e.g.*, ISCOM®, DETOX™, SAF®, Freund's adjuvant, Alum, Saponin, among others.

In another embodiment, the present invention provides methods for preparing monoclonal antibodies against the antigens encoded by the DNA sequences disclosed in the examples which are expressed specifically by certain malignant tissues including colon or colorectal tumor tissues. Monoclonal antibodies are produced by conventional methods and include human monoclonal antibodies, humanized monoclonal antibodies, chimeric monoclonal antibodies, single chain antibodies, including scFv's and antigen-binding antibody fragments such as Fabs, 2 Fabs, and Fab' fragments. Methods for the preparation of monoclonal antibodies and fragments thereof, for example by pepsin or papain-mediated cleavage, are well known in the art. In general, an appropriate (non-homologous) host is immunized with the subject colon cancer antigens, immune cells are isolated from the host and used to prepare hybridomas. Monoclonal antibodies that specifically bind to either of such antigens are identified by routine screening techniques. Useful monoclonal antibodies typically bind the target antigens with high affinity, *e.g.*, possess a binding affinity ( $K_d$ ) on the order of  $10^{-6}$  to  $10^{-10}$  M.

Monoclonal antibodies and fragments of the invention are useful for anti-tumor immunotherapy. Optionally, therapeutic effector moieties (*e.g.*, radiolabels, cytotoxins, therapeutic enzymes, agents that induce apoptosis) can be attached to the antibodies to provide for targeted cytotoxicity, *i.e.*, killing of human colon tumor cells. Given the fact that the subject genes are apparently not significantly expressed by many normal tissues this should not result in significant adverse side effects (toxicity to non-target tissues).

Antibodies and/or antibody fragments are administered to a subject in labeled or unlabeled form, alone or in combination with other therapeutics, such as chemotherapeutics such as progestin, EGFR, TAXOL®, and the like. The administered composition can include a pharmaceutically acceptable carrier, and optionally adjuvants, stabilizers, *etc.*, used in antibody compositions for therapeutic use.

The present invention also provides diagnostic methods for detection of the colon or colorectal tumor-specific genes disclosed herein. Diagnostic methods include detecting the expression of one or more of these genes at the DNA level or at the protein level. Patients who test positive for the disclosed tumor-specific genes diagnosed are identified as having or being at increased risk of developing colon cancer. Additionally, the levels of antigen expression can be useful in determining patient status, *i.e.*, how far the disease has advanced.

For example, the expression or expression level of a tumor-specific gene can indicate a particular stage of tumor progression.

At the DNA level, gene expression is detected by known DNA detection methods, including but not limited to Northern blot hybridization, strand displacement amplification (SDA), catalytic hybridization amplification (CHA), PCR amplification (for example, using primers corresponding to the novel genes disclosed herein), and other known DNA detection methods. For example, the presence or absence of cancer associated with the genes disclosed herein can be determined based on whether PCR products are obtained, and the level of expression. Expression levels can also be monitored to determine the prognosis of a colon cancer patient as the levels of expression of the PCR product likely increase as the disease progresses. Suitable controls and quantification is are performed for diagnostic methods as known in the art.

At the protein level, the status of a subject to be tested for colon cancer, or other cancer associated by overexpression of a gene disclosed herein, can be evaluated by testing biological fluids, such as blood, urine, colon tissue, with an antibody or antibodies or fragment that specifically binds to the novel colon tumor antigens disclosed herein. Methods of using antibodies to detect antigen expression are well known and include ELISA, competitive binding assays, and the like. Representative assays use an antibody or antibody fragment that specifically binds the target antigen directly or indirectly bound to a label that provides for detection, for example, a radiolabel, an enzyme, or a fluorophore.

As noted, the present invention provides novel genes and corresponding antigens that correlate to human colon cancer. The present invention also embraces variants thereof. By "variants" is intended sequences that are at least 75% identical thereto, for example at least 85% identical, or at least 90% identical when these DNA sequences are aligned to the subject DNAs or a fragment thereof having a size of at least 50 nucleotides. Representative variants include allelic variants.

The present invention also provides primers for amplification of nucleic acids encoding the subject novel genes or a portion thereof, which are present in a biological sample, for example, an mRNA library obtained from a desired cell source, including human colon cell or tissue samples. Typically, such primers are about 12 to 50 nucleotides in length and are constructed such that they provide for amplification of the entire or most of the target gene.

The present invention further provides antigens encoded by the disclosed DNAs or fragments thereof that bind to or elicit antibodies specific to the full-length antigens. Typically, such fragments are at least 10 amino acids in length, more typically at least 25 amino acids in length.

5           The colon or colorectal tumor-specific genes of the invention are expressed in a majority of colon tumor samples tested. Some of these genes are also upregulated in other cancers. Thus, the present invention further contemplates identification of other cancers wherein the expression of the disclosed genes or variants thereof correlate to a cancer or an increased likelihood of cancer, for example breast, pancreas, lung or colon cancers. Also  
10       provided are compositions and methods to detect and treat such cancers.

          “Isolated” refers to any human protein that is not in its normal cellular milieu. This includes by way of example compositions comprising recombinant protein, pharmaceutical compositions comprising purified protein, diagnostic compositions comprising purified protein, and isolated protein compositions comprising protein. In representative  
15       embodiments of the invention, an isolated protein comprises a substantially pure protein, in that it is substantially free of other proteins, for example, at least 90% pure, that comprises the amino acid sequence disclosed herein or natural homologues or mutants having essentially the same sequence. A naturally occurring mutant might be found, for instance, in tumor cells expressing a gene encoding a mutated protein sequence.

20       “Native human protein” refers to a protein that comprises the amino acid sequence of the protein expressed in its endogenous environment, *i.e.*, a human colon or colorectal tumor tissue.

          “Native non-human primate protein” refers to a protein that is a non-human primate homologue of the protein having the amino acid sequence discussed in the examples. Given  
25       the phylogenetic closeness of humans to other primates, it is anticipated that human and non-human proteins expressed by the genes disclosed in the examples have non-human primate counterparts that possess amino acid sequences that are highly similar, such as 95% sequence identity or higher.

          “Isolated human or non-human primate nucleic acid molecule or sequence” refers to a  
30       nucleic acid molecule that encodes human protein which is not in its normal human cellular milieu, *e.g.*, is not comprised in the human or non-human primate chromosomal DNA. This includes by way of example vectors that comprise a nucleic acid molecule, a probe that

comprises a gene nucleic acid sequence directly or indirectly attached to a detectable moiety, *e.g.* a fluorescent or radioactive label, or a DNA fusion that comprises a nucleic acid molecule encoding a colon antigen according to the invention fused at its 5' or 3' end to a different DNA, *e.g.* a promoter or a DNA encoding a detectable marker or effector moiety.

5 Representative nucleic acid sequence encoding human proteins are disclosed herein. Also included are natural homologues or mutants having substantially the same sequence. Naturally occurring homologues that are degenerate would encode the same protein as discussed herein in the examples, but would include nucleotide differences that do not change the corresponding amino acid sequence. Naturally occurring mutants might be found in  
10 tumor cells, wherein such nucleotide differences result in a mutant protein. Naturally occurring homologues containing conservative substitutions are also encompassed.

"Variant of human or non-human primate protein" refers to a protein possessing an amino acid sequence that possess at least 90% sequence identity, such as at least 91% sequence identity, or at least 92% sequence identity, or at least 93% sequence identity, or at  
15 least 94% sequence identity, or at least 95% sequence identity, or at least 96% sequence identity, or at least 97% sequence identity, or at least 98% sequence identity, and including at least 99% sequence identity, to the corresponding native human or non-human primate protein wherein sequence identity is as defined herein. Preferably, a variant possesses at least one biological property in common with the human or non-human protein.

20 "Variant of human or non-human primate nucleic acid molecule or sequence" refers to a nucleic acid sequence that possesses at least 90% sequence identity, such as at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98% sequence identity, and including at least 99% sequence identity, to the corresponding native human or non-human primate nucleic acid sequence, wherein  
25 "sequence identity" is as defined herein.

"Fragment of human or non-human primate nucleic acid molecule or sequence" refers to a nucleic acid sequence corresponding to a portion of the native human nucleic acid sequence discussed herein in the examples or a primate native non-human homolog molecule, wherein said portion is at least about 50 nucleotides in length, or 100, for example, at least  
30 200 or 300 nucleotides in length.

"Antigenic fragments of colon or colorectal" refer to polypeptides corresponding to a fragment of colon antigen encoded by any of the genes disclosed herein or a variant or

homologue thereof that when used itself or attached to an immunogenic carrier that elicits antibodies that specifically bind the protein. Typically, antigenic fragments are at least 20 amino acids in length.

Sequence identity or percent identity is intended to mean the percentage of the same  
5 residues shared between two sequences, referenced to the human DNA or amino acid  
sequences disclosed herein, when the two sequences are aligned using the Clustal method  
[Higgins et al, Cabios 8:189-191 (1992)] of multiple sequence alignment in the Lasergene  
biocomputing software (DNASTAR, INC. of Madison, Wisconsin). In this method, multiple  
10 alignments are carried out in a progressive manner, in which larger and larger alignment  
groups are assembled using similarity scores calculated from a series of pairwise alignments.  
Optimal sequence alignments are obtained by finding the maximum alignment score, which  
is the average of all scores between the separate residues in the alignment, determined from a  
residue weight table representing the probability of a given amino acid change occurring in  
15 two related proteins over a given evolutionary interval. Penalties for opening and  
lengthening gaps in the alignment contribute to the score. The default parameters used with  
this program are as follows: gap penalty for multiple alignment=10; gap length penalty for  
multiple alignment=10; k-tuple value in pairwise alignment=1; gap penalty in pairwise  
alignment=3; window value in pairwise alignment=5; diagonals saved in pairwise  
alignment=5. The residue weight table used for the alignment program is PAM250  
20 [Dayhoff et al., in Atlas of Protein Sequence and Structure, Dayhoff, Ed., NDRF, Washington,  
Vol. 5, suppl. 3, p. 345, (1978)].

Percent conservation is calculated from the above alignment by adding the percentage  
of identical residues to the percentage of positions at which the two residues represent a  
conservative substitution (defined as having a log odds value of greater than or equal to 0.3 in  
25 the PAM250 residue weight table). Conservation is referenced to a human gene of the  
invention when determining percent conservation with a non-human gene and when  
determining percent conservation. Conservative amino acid changes satisfying this  
requirement include: R-K; E-D, Y-F, L-M; V-I, Q-H.

### 30 *Polypeptide Fragments*

The invention provides polypeptide fragments of the disclosed proteins. Polypeptide  
fragments of the invention can comprise at least 8 amino acid residues, such as at least 25 or

at least 50 amino acid residues of human or non-human primate gene according to the invention or an analogue thereof. Polypeptide fragments can also comprise at least 75, 100, 125, 150, 175, 200, 225, 250, or 275 residues of the polypeptide encoded by gene the subject genes which are specifically expressed by certain human colon or colorectal as well as some other tumor tissues. In one embodiment of the invention, a protein fragment can also comprise a majority of the native protein colon or colorectal protein, *i.e.* at least about 100 contiguous residues of the native colon or colorectal protein antigen.

### *Biologically Active Variants*

The invention also encompasses biologically active mutants of protein colon or colorectal proteins according to the invention, which comprise an amino acid sequence that is at least 80%, for example, 90% or 95-99% similar to the subject tumor-associated proteins.

Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity can be found using computer programs well known in the art, such as DNASTAR software. Protein variants can include conoservative amino acid changes, *i.e.*, substitutions of similarly charged or uncharged amino acids. A conservative amino acid change involves substitution of one of a family of amino acids which are related in their side chains. Naturally occurring amino acids are generally divided into four families: acidic (aspartate, glutamate), basic (lysine, arginine, histidine), non-polar (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), and uncharged polar (glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine) amino acids. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids.

A subset of mutants, called muteins, is a group of polypeptides in which neutral amino acids, such as serines, are substituted for cysteine residues which do not participate in disulfide bonds. These mutants may be stable over a broader temperature range than native secreted proteins. See Mark *et al.*, U.S. Patent 4,959,314.

It is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid can be made without affecting the biological properties of the resulting secreted protein or polypeptide variant.

Human or non-human primate protein variants include glycosylated forms,

aggregative conjugates with other molecules, and covalent conjugates with unrelated chemical moieties. Also, protein variants also include allelic variants, species variants, and muteins. Truncations or deletions of regions which do not affect the differential expression of the protein gene are also variants. Covalent variants can be prepared by linking  
5 functionalities to groups which are found in the amino acid chain or at the N- or C-terminal residue, as is known in the art.

Some amino acid sequence of the proteins of the invention can be varied without significant effect on the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there are critical areas on the protein which  
10 determine activity. In general, it is possible to replace residues that form the tertiary structure, provided that residues performing a similar function are used. Numerous substitutions at non-critical regions of the protein are well tolerated. The replacement of amino acids can also change the selectivity of binding to cell surface receptors. Ostade et al., *Nature* 361:266-268 (1993) describes certain mutations resulting in selective binding of TNF-  
15 alpha to only one of the two known types of TNF receptors. Thus, the polypeptides of the present invention can include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation.

The invention further includes variations of the protein subject colon or colorectal which show comparable expression patterns or which include antigenic regions. Protein  
20 mutants include deletions, insertions, inversions, repeats, and type substitutions. Guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U., et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990).

For example, charged amino acids can be substituted with another charged amino  
25 acid, or with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the disclosed protein. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard et al., *Clin. Exp. Immunol.* 2:331-340 (1967);  
30 Robbins et al., *Diabetes* 36:838-845 (1987); Cleland et al., *Crit. Rev. Therapeutic Drug Carrier Systems* 10:307-377 (1993)).

Amino acids in the polypeptides of the present invention that are essential for function



can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244: 1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as binding to a natural or synthetic binding partner. Sites that are critical for ligand-receptor binding can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith et al., *J Mol. Biol.* 224:899-904 (1992) and de Vos et al. *Science* 255: 306-312 (1992)).

Conservative amino acid substitutions often do not significantly affect the folding or activity of the protein. A skilled artisan could determine an appropriate number and nature of amino acid substitutions based on factors as described above. Generally speaking, the number of substitutions for any given polypeptide are fewer than 50, 40, 30, 25, 20, 15, 10, 5 or 3 residues.

### *Fusion Proteins*

Fusion proteins comprising proteins or polypeptide fragments of the subject colon or colorectal proteins can also be constructed. Fusion proteins are useful for generating antibodies against amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins which interact with a protein of the invention or which interfere with its biological function. Physical methods, such as protein affinity chromatography, or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can also be used for this purpose. The foregoing can also be adapted as a screening technique. Fusion proteins comprising a signal sequence and/or a transmembrane domain of a protein according to the invention or a fragment thereof can be used to target other protein domains to cellular locations in which the domains are not normally found, such as bound to a cellular membrane or secreted extracellularly.

A fusion protein comprises two protein segments fused together by means of a peptide bond. Amino acid sequences for use in fusion proteins of the invention can utilize any of the amino acid sequences or encoded by the nucleotide sequences disclosed herein, or can be prepared from biologically active variants or fragment of said protein sequence, such as those described above. The first protein segment can consist of a full-length protein or a variant or fragment thereof. These fragments can range in size from about 8 amino acids up

to the full length of the protein.

The second protein segment can be a full-length protein or a polypeptide fragment. Proteins commonly used in fusion protein construction include  $\beta$ -galactosidase,  $\beta$ -glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue  
5 fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags can be used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA  
10 binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions.

These fusions can be made, for example, by covalently linking two protein segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises  
15 a coding sequence encoding an amino acid sequence according to the invention in proper reading frame with a nucleotide encoding the second protein segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies that supply research labs with tools for experiments, including, for example, Promega Corporation (Madison, WI), Stratagene (La Jolla, CA),  
20 Clontech (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Proteins, fusion proteins, or polypeptides of the invention can be produced by recombinant DNA methods. For production of recombinant proteins, fusion proteins, or  
25 polypeptides, a sequence listing encoding one of the subject colon or colorectal proteins can be expressed in prokaryotic or eukaryotic host cells using expression systems known in the art. These expression systems include bacterial, yeast, insect, and mammalian cells.

The resulting expressed protein can then be purified from the culture medium or from extracts of the cultured cells using purification procedures known in the art. For example, for  
30 proteins fully secreted into the culture medium, cell-free medium can be diluted with sodium acetate and contacted with a cation exchange resin, followed by hydrophobic interaction chromatography. Using this method, the desired protein or polypeptide is typically greater

than 95% pure. Further purification can be undertaken, using, for example, any of the techniques listed above.

Proteins can be further modified, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain a functional protein. Covalent attachments can be made using known chemical or enzymatic methods.

Human or non-human primate proteins according to the invention or polypeptide of the invention can also be expressed in cultured host cells in a form that facilitates purification. For example, a protein or polypeptide can be expressed as a fusion protein comprising, for example, maltose binding protein, glutathione-S-transferase, or thioredoxin, and purified using a commercially available kit. Kits for expression and purification of such fusion proteins are available from companies such as New England BioLabs, Pharmacia, and Invitrogen. Proteins, fusion proteins, or polypeptides can also be tagged with an epitope, such as a "Flag" epitope (Kodak), and purified using an antibody which specifically binds to that epitope.

The coding sequence disclosed herein can also be used to construct transgenic animals, such as mice, rats, guinea pigs, cows, goats, pigs, or sheep. Female transgenic animals can then produce proteins, polypeptides, or fusion proteins of the invention in their milk. Methods for constructing such animals are known and widely used in the art.

Alternatively, synthetic chemical methods, such as solid phase peptide synthesis, can be used to synthesize a secreted protein or polypeptide. General means for the production of peptides, analogs or derivatives are outlined in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins -- A Survey of Recent Developments, B. Weinstein, ed. (1983). Substitution of D-amino acids for the normal L-stereoisomer can be carried out to increase the half-life of the molecule.

Typically, homologous polynucleotide sequences can be confirmed by hybridization under stringent conditions, as is known in the art. For example, using the following wash conditions: 2X SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2X SSC, 0.1% SDS, 50°C once, 30 minutes; then 2X SSC, room temperature twice, 10 minutes each, homologous sequences can be identified which contain at most about 25-30% base pair mismatches. Homologous nucleic acids can contain 15-25% base pair mismatches or fewer, for example about 5-15% base pair mismatches.

The invention also provides polynucleotide probes which can be used to detect complementary nucleotide sequences, for example, in hybridization protocols such as Northern or Southern blotting or *in situ* hybridizations. Polynucleotide probes of the invention comprise at least 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, or 40 or more contiguous nucleotides of the gene A and gene B nucleic acid sequences provided herein. Polynucleotide probes of the invention can comprise a detectable label, such as a radioisotopic, fluorescent, enzymatic, or chemiluminescent label.

Isolated genes corresponding to the cDNA sequences disclosed herein are also provided. Standard molecular biology methods can be used to isolate the corresponding genes using the cDNA sequences provided herein. These methods include preparation of probes or primers based on the disclosed sequences for use in identifying or amplifying the genes from mammalian, including human, genomic libraries or other sources of human genomic DNA.

Polynucleotide molecules of the invention can also be used as primers to obtain additional copies of the polynucleotides, using polynucleotide amplification methods. Polynucleotide molecules can be propagated in vectors and cell lines using techniques well known in the art. Polynucleotide molecules can be on linear or circular molecules. They can be on autonomously replicating molecules or on molecules without replication sequences. They can be regulated by their own or by other regulatory sequences, as is known in the art.

#### *Polynucleotide Constructs*

Polynucleotide molecules comprising the coding sequences disclosed herein can be used in a polynucleotide construct, such as a DNA or RNA construct. Polynucleotide molecules of the invention can be used, for example, in an expression construct to express all or a portion of a protein, variant, fusion protein, or single-chain antibody in a host cell. An expression construct comprises a promoter which is functional in a chosen host cell. The skilled artisan can readily select an appropriate promoter from the large number of cell type-specific promoters known and used in the art. The expression construct can also contain a transcription terminator which is functional in the host cell. The expression construct comprises a polynucleotide segment which encodes all or a portion of the desired protein. The polynucleotide segment is located downstream from the promoter. Transcription of the polynucleotide segment initiates at the promoter. The expression construct can be linear or

circular and can contain sequences, if desired, for autonomous replication.

Also included are polynucleotide molecules comprising human or non-human primate gene promoter and UTR sequences, operably linked to either protein coding sequences or other sequences encoding a detectable or selectable marker. Promoter and/or UTR-based constructs are useful for studying the transcriptional and translational regulation of protein expression, and for identifying activating and/or inhibitory regulatory proteins.

### Host Cells

An expression construct can be introduced into a host cell. The host cell comprising the expression construct can be any suitable prokaryotic or eukaryotic cell. Expression systems in bacteria include those described in Chang *et al.*, *Nature* 275:615 (1978); Goeddel *et al.*, *Nature* 281: 544 (1979); Goeddel *et al.*, *Nucleic Acids Res.* 8:4057 (1980); EP 36,776; U.S. 4,551,433; deBoer *et al.*, *Proc. Natl. Acad. Sci. USA* 80: 21-25 (1983); and Siebenlist *et al.*, *Cell* 20: 269 (1980).

Expression systems in yeast include those described in Hinnen *et al.*, *Proc. Natl. Acad. Sci. USA* 75: 1929 (1978); Ito *et al.*, *J. Bacteriol.* 153: 163 (1983); Kurtz *et al.*, *Mol. Cell. Biol.* 6: 142 (1986); Kunze *et al.*, *J. Basic Microbiol.* 25: 141 (1985); Gleeson *et al.*, *J. Gen. Microbiol.* 132: 3459 (1986); Roggenkamp *et al.*, *Mol. Gen. Genet.* 202: 302 (1986); Das *et al.*, *J. Bacteriol.* 158: 1165 (1984); De Louvencourt *et al.*, *J. Bacteriol.* 154:737 (1983); Van den Berg *et al.*, *Bio/Technology* 8: 135 (1990); Kunze *et al.*, *J. Basic Microbiol.* 25: 141 (1985); Cregg *et al.*, *Mol. Cell. Biol.* 5: 3376 (1985); U.S. 4,837,148; U.S. 4,929,555; Beach and Nurse, *Nature* 300: 706 (1981); Davidow *et al.*, *Curr. Genet.* 10: 380 (1985); Gaillardin *et al.*, *Curr. Genet.* 10: 49 (1985); Ballance *et al.*, *Biochem. Biophys. Res. Commun.* 112: 284-289 (1983); Tilburn *et al.*, *Gene* 26: 205-22 (1983); Yelton *et al.*, *Proc. Natl. Acad. Sci. USA* 81: 1470-1474 (1984); Kelly and Hynes, *EMBO J.* 4: 475479 (1985); EP 244,234; and WO 91/00357.

Expression of heterologous genes in insects can be accomplished as described in U.S. 4,745,051; Friesen *et al.* (1986) "The Regulation of Baculovirus Gene Expression" in: THE MOLECULAR BIOLOGY OF BACULOVIRUSES (W. Doerfler, ed.); EP 127,839; EP 155,476; Vlak *et al.*, *J. Gen. Virol.* 69: 765-776 (1988); Miller *et al.*, *Ann. Rev. Microbiol.* 42: 177 (1988); Carbonell *et al.*, *Gene* 73: 409 (1988); Maeda *et al.*, *Nature* 315: 592-594 (1985); Lebacqz-Verheyden *et al.*, *Mol. Cell Biol.* 8: 3129 (1988); Smith *et al.*, *Proc. Natl. Acad. Sci.*

USA 82: 8404 (1985); Miyajima *et al.*, *Gene* 58: 273 (1987); and Martin *et al.*, *DNA* 7:99 (1988). Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts are described in Luckow *et al.*, *Bio/Technology* (1988) 6: 47-55, Miller *et al.*, in GENETIC ENGINEERING (Setlow, J.K. *et al.* eds.), Vol. 8, pp. 277-279 (Plenum Publishing, 1986); and Maeda *et al.*, *Nature*, 315: 592-594 (1985).

Mammalian expression can be accomplished as described in Dijkema *et al.*, *EMBO J.* 4: 761(1985); Gorman *et al.*, *Proc. Natl. Acad. Sci. USA* 79: 6777 (1982b); Boshart *et al.*, *Cell* 41: 521 (1985); and U.S. 4,399,216. Other features of mammalian expression can be facilitated as described in Ham and Wallace, *Meth. Enz.* 58: 44 (1979); Barnes and Sato, *Anal. Biochem.* 102: 255 (1980); U.S. 4,767,704; U.S. 4,657,866; U.S. 4,927,762; U.S. 4,560,655; WO 90/103430, WO 87/00195, and U.S. RE 30,985.

Expression constructs can be introduced into host cells using any technique known in the art. These techniques include transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and calcium phosphate-mediated transfection.

Expression of an endogenous gene encoding a protein of the invention can also be manipulated by introducing by homologous recombination a DNA construct comprising a transcription unit in frame with the endogenous gene, to form a homologously recombinant cell comprising the transcription unit. The transcription unit comprises a targeting sequence, a regulatory sequence, an exon, and an unpaired splice donor site. The new transcription unit can be used to turn the endogenous gene on or off as desired. This method of affecting endogenous gene expression is taught in U.S. Patent 5,641,670.

The targeting sequence is a segment of at least 10, 12, 15, 20, or 50 contiguous nucleotides of the nucleotide sequences disclosed herein. The transcription unit is located upstream to a coding sequence of the endogenous gene. The exogenous regulatory sequence directs transcription of the coding sequence of the endogenous gene.

Human or non-human primate protein can also include hybrid and modified forms thereof including fusion proteins, fragments and hybrid and modified forms in which certain amino acids have been deleted or replaced, modifications such as where one or more amino acids have been changed to a modified amino acid or unusual amino acid.

Also included within the meaning of substantially homologous is any human or non-

human primate protein which shows cross-reactivity with antibodies to a gene described herein or whose encoding nucleotide sequences including genomic DNA, mRNA or cDNA are isolated through hybridization with the complementary sequence of genomic or subgenomic nucleotide sequences or cDNA of a gene disclosed herein or a fragment thereof.

5 Degenerate DNA sequences that encode human or non-human primate proteins are also included within the present invention as are allelic variants of.

Colon or colorectal proteins of the invention can be prepared using recombinant DNA techniques. By "pure form" or "purified form" or "substantially purified form" it is meant that a protein composition is substantially free of other proteins which are not protein.

10 The present invention also includes therapeutic or pharmaceutical compositions comprising human or non-human primate proteins, fragments or variants according to the invention in an effective amount for treating patients with disease, and a method comprising administering a therapeutically effective amount of a protein according to the invention. These compositions and methods are useful for treating cancers associated with a protein  
15 according to the invention, *e.g.* colon cancer. One skilled in the art can readily use a variety of assays known in the art to determine whether a protein according to the invention would be useful in promoting survival or functioning in a particular cell type.

In certain circumstances, it may be desirable to modulate or decrease the amount of the subject colon or colorectal protein expressed. Thus, in another aspect of the present  
20 invention, anti-sense oligonucleotides can be made specific to genes disclosed herein and a method utilized for diminishing the level of expression a protein according to the invention by a cell comprising administering one or more gene anti-sense oligonucleotides. By gene specific anti-sense oligonucleotides reference is made to oligonucleotides that have a nucleotide sequence that interacts through base pairing with a specific complementary  
25 nucleic acid sequence involved in the expression of a gene according to the invention that the expression of the gene is reduced. Nucleic acids involved in the expression of the subject gene include genomic DNA and mRNA that encode a colon or colorectal gene disclosed herein. This genomic DNA molecule can comprise regulatory regions of the gene, or the coding sequence for mature gene encoded by the gene.

30 The term complementary to a nucleotide sequence in the context of antisense oligonucleotides and methods therefor means sufficiently complementary to such a sequence as to allow hybridization to that sequence in a cell, *i.e.*, under physiological conditions. The

antisense oligonucleotides can comprise a sequence containing from about 8 to about 100 nucleotides, including antisense oligonucleotides that comprise from about 15 to about 30 nucleotides. The antisense oligonucleotides can also contain a variety of modifications that confer resistance to nucleolytic degradation such as, for example, modified internucleoside  
5 linkages [Uhlmann and Peyman, *Chemical Reviews* 90:543-548 (1990); Schneider and Banner, *Tetrahedron Lett.* 31:335, (1990) which are incorporated by reference], modified nucleic acid bases as disclosed in 5,958,773 and patents disclosed therein, and/or sugars and the like.

Any modifications or variations of the antisense molecule which are known in the art to be broadly applicable to antisense technology are included within the scope of the  
10 invention. Representative modifications include preparation of phosphorus-containing linkages as disclosed in U.S. Patents 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361, 5,625,050 and 5,958,773.

The antisense compounds of the invention can include modified bases. The antisense oligonucleotides of the invention can also be modified by chemically linking the  
15 oligonucleotide to one or more moieties or conjugates to enhance the activity, cellular distribution, or cellular uptake of the antisense oligonucleotide. Representative moieties or conjugates include lipids such as cholesterol, cholic acid, thioether, aliphatic chains, phospholipids, polyamines, polyethylene glycol (PEG), palmityl moieties, and others as disclosed in, for example, U.S. Patents 5,514,758, 5,565,552, 5,567,810, 5,574,142,  
20 5,585,481, 5,587,371, 5,597,696 and 5,958,773.

Chimeric antisense oligonucleotides are also within the scope of the invention, and can be prepared from the present inventive oligonucleotides using the methods described in, for example, U.S. Patents 5,013,830, 5,149,797, 5,403,711, 5,491,133, 5,565,350, 5,652,355, 5,700,922 and 5,958,773.

Select of optimal antisense molecules for particular targets typically involves routine screening of a number of candidate molecules. An antisense molecule can be targeted to an accessible, or exposed, portion of the target RNA molecule. Although in some cases information is available about the structure of target mRNA molecules, the current approach to inhibition using antisense is via experimentation. mRNA levels in the cell can be  
25 measured routinely in treated and control cells by reverse transcription of the mRNA and assaying the cDNA levels. The biological effect can be determined routinely by measuring cell growth or viability as is known in the art.  
30



Measuring the specificity of antisense activity by assaying and analyzing cDNA levels is an art-recognized method of validating antisense results. It has been suggested that RNA from treated and control cells should be reverse-transcribed and the resulting cDNA populations analyzed. [Branch, A. D., *T.I.B.S.* 23:45-50 (1998)].

5           The therapeutic or pharmaceutical compositions of the present invention can be administered by any suitable route known in the art including for example intravenous, subcutaneous, intramuscular, transdermal, intrathecal or intracerebral. Administration can be either rapid as by injection or over a period of time as by slow infusion or administration of slow release formulation.

10           Additionally, a human or non-human primate protein according to the invention can also be linked or conjugated with agents that provide desirable pharmaceutical or pharmacodynamic properties. For example, the protein can be coupled to any substance known in the art to promote penetration or transport across the blood-brain barrier such as an antibody to the transferrin receptor, and administered by intravenous injection (see, for  
15           example, Friden et al., *Science* 259:373-377 (1993) which is incorporated by reference). Furthermore, the subject protein can be stably linked to a polymer such as polyethylene glycol to obtain desirable properties of solubility, stability, half-life and other pharmaceutically advantageous properties. [See, for example, Davis et al., *Enzyme Eng.* 4:169-73 (1978); Buruham, *Am. J. Hosp. Pharm.* 51:210-218 (1994) which are incorporated  
20           by reference].

          The compositions are usually employed in the form of pharmaceutical preparations, which are made in a manner well known in the pharmaceutical art. See, e.g. Remington Pharmaceutical Science, 18th Ed., Merck Publishing Co. Eastern PA, (1990). Physiological saline solutions can be used, as well as other pharmaceutically acceptable carriers such as  
25           physiological concentrations of other non-toxic salts, five percent aqueous glucose solution, sterile water and the like. Compositions of the invention can also include a suitable buffer. Optionally, such solutions can be lyophilized and stored in a sterile ampoule ready for reconstitution by the addition of sterile water for ready injection. The primary solvent can be aqueous or alternatively non-aqueous. The subject human or primate protein, fragment or  
30           variant thereof can also be incorporated into a solid or semi-solid biologically compatible matrix which can be implanted into tissues requiring treatment.

          The carrier can also contain other pharmaceutically-acceptable excipients for

modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier can contain still other pharmaceutically-acceptable excipients for modifying or maintaining release or absorption or penetration across the blood-brain barrier. Excipients are those substances usually and customarily employed to formulate dosages for parenteral administration in either unit dosage or multi-dose form or for direct infusion into the cerebrospinal fluid by continuous or periodic infusion.

Dose administration can be repeated depending upon the pharmacokinetic parameters of the dosage formulation and the route of administration used.

It is also contemplated that certain formulations containing a protein according to the invention or variant or fragment thereof are to be administered orally. Protein formulations can be encapsulated and formulated with suitable carriers in solid dosage forms. Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, gelatin, syrup, methyl cellulose, methyl- and propylhydroxybenzoates, talc, magnesium, stearate, water, mineral oil, and the like. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions can be formulated so as to provide rapid, sustained, or delayed release of the active ingredients after administration to the patient by employing procedures well known in the art. The formulations can also contain substances that diminish proteolytic degradation and promote absorption such as, for example, surface active agents.

The specific dose is calculated according to the approximate body weight or body surface area of the patient or the volume of body space to be occupied. The dose also depends on the particular route of administration selected. Further refinement of the calculations necessary to determine the appropriate dosage for treatment is routinely made by those of ordinary skill in the art. Following a review of the present disclosure, an effective dosage can be determined without undue experimentation. Exact dosages are determined in conjunction with standard dose-response studies. The amount of the composition actually administered can be determined by a practitioner, in the light of the relevant circumstances including the condition or conditions to be treated, the choice of composition to be administered, the age, weight, and response of the individual patient, the severity of the

patient's symptoms, and the chosen route of administration.

In one embodiment, a protein of the present invention is therapeutically administered by implanting into patients vectors or cells capable of producing a biologically-active form of the protein or a precursor of the protein, *i.e.*, a molecule that can be readily converted to a biological-active form of the by the body. For example, cells that secrete the protein can be encapsulated into semipermeable membranes for implantation into a patient. The cells can be cells that normally express the protein or a precursor thereof or the cells can be transformed to express the protein or a precursor thereof. For human subjects, a human protein can be used, or a non-human primate protein homolog of a human protein can be used.

In a number of circumstances it would be desirable to determine the levels of protein or corresponding mRNA encoding a protein according to the invention in a patient. The identification of the subject genes which are specifically expressed by colon or colorectal tumors suggests these proteins are expressed at different levels during some diseases, *e.g.*, cancers, provides the basis for the conclusion that the presence of these proteins serves a normal physiological function related to cell growth and survival. Endogenously produced human colon or colorectal antigen according to the invention may also play a role in certain disease conditions.

The term "detection" as used herein in the context of detecting the presence of a cancer gene according to the invention in a patient is intended to include the determining of the amount of protein according to the invention or the ability to express an amount of this protein in a patient, the estimation of prognosis in terms of probable outcome of a disease and prospect for recovery, the monitoring of these protein levels over a period of time as a measure of status of the condition, and the monitoring of colon or colorectal protein according to the invention for determining an effective therapeutic regimen for the patient, *e.g.* one with colon cancer.

To detect the presence of a gene according to the invention in a patient, a sample is obtained from the patient. The sample can be a tissue biopsy sample or a sample of blood, plasma, serum, CSF or the like. It has been found that the subject genes are expressed at high levels in some cancers, *e.g.*, colon or colorectal cancers. Samples for detecting protein can be taken from these tissue. When assessing peripheral levels of protein, a sample of blood, plasma or serum can be used. When assessing the levels of protein in the central nervous system, samples can be obtained from cerebrospinal fluid or neural tissue.

In some instances, it is desirable to determine whether a gene according to the invention is intact in the patient or in a tissue or cell line within the patient. By an intact gene, it is meant that there are no alterations in the gene such as point mutations, deletions, insertions, chromosomal breakage, chromosomal rearrangements and the like wherein such alteration might alter the production of gene or alter its biological activity, stability or the like to lead to disease processes. Thus, in one embodiment of the present invention a method is provided for detecting and characterizing any alterations in the gene. The method comprises providing an oligonucleotide that contains the gene corresponding cDNA, genomic DNA or a fragment thereof or a derivative thereof. By a derivative of an oligonucleotide, it is meant that the derived oligonucleotide is substantially the same as the sequence from which it is derived in that the derived sequence has sufficient sequence complementarity to the sequence from which it is derived to hybridize specifically to the gene. A nucleic acid of the invention can be isolated, chemically synthesized, of recombinantly produced (*e.g.*, using *in vitro* DNA replication, reverse transcription, or transcription).

Typically, patient genomic DNA is isolated from a cell sample from the patient and digested with one or more restriction endonucleases such as, for example, TaqI and AluI. Using the Southern blot protocol, which is well known in the art, this assay determines whether a patient or a particular tissue in a patient has an intact gene according to the invention or a gene abnormality.

Hybridization to a gene according to the invention would involve denaturing the chromosomal DNA to obtain a single-stranded DNA; contacting the single-stranded DNA with a gene probe associated with the gene sequence; and identifying the hybridized DNA-probe to detect chromosomal DNA containing at least a portion of a human gene according to the invention.

The term "probe" as used herein refers to a structure comprised of a polynucleotide that forms a hybrid structure with a target sequence, due to complementarity of probe sequence with a sequence in the target region. Oligomers suitable for use as probes typically contain at least about 8-12 contiguous nucleotides which are complementary to the targeted sequence, for example 20 nucleotides.

Probes of the present invention can be DNA or RNA oligonucleotides and can be made by any method known in the art such as, for example, excision, transcription or chemical synthesis. Probes can be labeled with any detectable label known in the art such as,

for example, radioactive or fluorescent labels or enzymatic marker. Labeling of the probe can be accomplished by any method known in the art such as by PCR, random priming, end labeling, nick translation or the like. Methods that do not employ a labeled probe can also be used to determine the hybridization. Representative techniques include Southern blotting, fluorescence *in situ* hybridization, and single-strand conformation polymorphism with PCR amplification.

Hybridization is typically carried out at about 25° - 45° C, or at about 32° -40° C, or at about 37° - 38° C. Hybridization can proceed for about 0.25 hour to about 96 hours, or from about 1 (one) hour to about 72 hours, or from about 4 hours to about 24 hours.

Gene abnormalities can also be detected by using the PCR method and primers that flank or lie within the particular gene. The PCR method is well known in the art. Briefly, this method is performed using two oligonucleotide primers which are capable of hybridizing to the nucleic acid sequences flanking a target sequence that lies within gene and amplifying the target sequence. The terms "oligonucleotide primer" as used herein refers to a short strand of DNA or RNA ranging in length from about 8 to about 30 bases. The upstream and downstream primers are typically from about 20 to about 30 base pairs in length and hybridize to the flanking regions for replication of the nucleotide sequence. The polymerization is catalyzed by a DNA-polymerase in the presence of deoxynucleotide triphosphates or nucleotide analogs to produce double-stranded DNA molecules. The double strands are then separated by any denaturing method including physical, chemical or enzymatic. Commonly, a method of physical denaturation is used involving heating the nucleic acid, typically to temperatures from about 80°C to 105°C for times ranging from about 1 to about 10 minutes. The process is repeated for the desired number of cycles.

The primers are selected to be substantially complementary to the strand of DNA being amplified. Therefore, the primers need not reflect the exact sequence of the template, but must be sufficiently complementary to selectively hybridize with the strand being amplified.

After PCR amplification, the DNA sequence comprising a gene of the invention or a fragment thereof is then directly sequenced and analyzed by comparison of the sequence with the sequences disclosed herein to identify alterations which might change activity or expression levels or the like.

In another embodiment, a method for detecting protein a colon according to the

invention is provided based upon an analysis of tissue expressing the gene. Certain tissues such as breast, lung, colon and others can be analyzed. The method comprises hybridizing a polynucleotide to mRNA from a sample of tissue that normally expresses the gene. The sample is obtained from a patient suspected of having an abnormality in the gene.

5 To detect the presence of mRNA encoding protein a colon or colorectal protein according to the invention is obtained from a patient. The sample can be from blood or from a tissue biopsy sample. The sample can be treated to extract the nucleic acids contained therein. The resulting nucleic acid from the sample is subjected to gel electrophoresis or other size separation techniques.

10 The mRNA of the sample is contacted with a DNA sequence serving as a probe to form hybrid duplexes. The use of a labeled probes as discussed above allows detection of the resulting duplex.

When using the cDNA encoding a colon or colorectal protein according to the invention or a derivative of the cDNA as a probe, high stringency conditions can be used in order to prevent false positives, that is the hybridization and apparent detection of the gene nucleotide sequences when in fact an intact and functioning gene is not present. When using sequences derived from the gene or cDNA, less stringent conditions could be used, however, are less preferred because of the likelihood of false positives. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, length of time and concentration of formamide. These factors are outlined in, for example, Sambrook et al. [Sambrook et al. (1989), *supra*].

15 In order to increase the sensitivity of the detection in a sample of mRNA encoding the protein, the technique of reverse transcription/ polymerization chain reaction (RT/PCR) can be used to amplify cDNA transcribed from mRNA encoding the protein. The method of RT/PCR is well known in the art, and can be performed as follows. Total cellular RNA is isolated by, for example, the standard guanidium isothiocyanate method and the total RNA is reverse transcribed. The reverse transcription method involves synthesis of DNA on a template of RNA using a reverse transcriptase enzyme and a 3' end primer. Typically, the primer contains an oligo(dT) sequence. The cDNA thus produced is then amplified using the PCR method and specific primers. [Belyavsky et al., *Nucl. Acid Res.* 17:2919-2932 (1989); Krug and Berger, *Methods in Enzymology*, 152:316-325, Academic Press, NY (1987) which are incorporated by reference].

The polymerase chain reaction method is performed as described above using two oligonucleotide primers that are substantially complementary to the two flanking regions of the DNA segment to be amplified. Following amplification, the PCR product is then electrophoresed and detected by ethidium bromide staining or by phosphoimaging.

5       The present invention further provides for methods to detect the presence of a colon or colorectal protein in a sample obtained from a patient. Any method known in the art for detecting proteins can be used. Representative methods include, but are not limited to immunodiffusion, immunoelectrophoresis, immunochemical methods, binder-ligand assays, immunohistochemical techniques, agglutination and complement assays. [*Basic and Clinical*  
10 *Immunology*, 217-262, Sites and Terr, eds., Appleton & Lange, Norwalk, CT, (1991), which is incorporated by reference]. For example, binder-ligand immunoassays can be used, which involve reacting antibodies with an epitope or epitopes of a colon protein of the invention and competitively displacing a labeled protein or derivative thereof.

As used herein, a derivative of a protein according to the invention is intended to  
15 include a polypeptide in which certain amino acids have been deleted or replaced or changed to modified or unusual amino acids wherein the derivative is biologically equivalent to the gene and wherein the polypeptide derivative cross-reacts with antibodies raised against the protein. By cross-reaction it is meant that an antibody reacts with an antigen other than the one that induced its formation.

20       Numerous competitive and non-competitive protein-binding immunoassays are well known in the art. Antibodies employed in such assays can be unlabeled, for example as used in agglutination tests, or labeled for use in a wide variety of assay methods. Labels that can be used include radionuclides, enzymes, fluorescers, chemilumescers, enzyme substrates or co-factors, enzyme inhibitors, particles, dyes and the like for use in radioimmunoassay (RIA),  
25 enzyme immunoassays, e.g., enzyme-linked immunosorbent assay (ELISA), fluorescent immunoassays and the like.

Polyclonal or monoclonal antibodies to the subject non-human primate or human proteins or according to the invention an epitope thereof can be made for use in immunoassays by any of a number of methods known in the art. By epitope reference is  
30 made to an antigenic determinant of a polypeptide. An epitope could comprise 3 amino acids in a spatial conformation which is unique to the epitope. Generally an epitope consists of at least 5 such amino acids. Methods of determining the spatial conformation of amino acids

are known in the art, and include, for example, x-ray crystallography and 2 dimensional nuclear magnetic resonance.

One approach for preparing antibodies to a protein is the selection and preparation of an amino acid sequence of all or part of the protein, chemically synthesizing the sequence and  
5 injecting it into an appropriate animal, typically a rabbit, hamster or a mouse.

Oligopeptides can be selected as candidates for the production of an antibody to the subject colon or colorectal protein based upon the oligopeptides lying in hydrophilic regions, which are thus likely to be exposed in the mature protein.

Additional oligopeptides can be determined using, for example, the Antigenicity  
10 Index, Welling, G.W. et al., *FEBS Lett.* 188:215-218 (1985), incorporated herein by reference.

In other embodiments of the present invention, humanized monoclonal antibodies are provided, wherein the antibodies are specific for a protein according to the invention. The phrase "humanized antibody" refers to an antibody derived from a non-human antibody,  
15 typically a mouse monoclonal antibody. Alternatively, a humanized antibody can be derived from a chimeric antibody that retains or substantially retains the antigen-binding properties of the parental, non-human, antibody but which exhibits diminished immunogenicity as compared to the parental antibody when administered to humans. The phrase "chimeric antibody," as used herein, refers to an antibody containing sequence derived from two  
20 different antibodies (*see, e.g.*, U.S. Patent No. 4,816,567) which typically originate from different species. Most typically, chimeric antibodies comprise human and murine antibody fragments generally human constant and mouse variable regions.

Because humanized antibodies are far less immunogenic in humans than the parental mouse monoclonal antibodies, they can be used for the treatment of humans with far less risk  
25 of anaphylaxis. Thus, these antibodies are useful in therapeutic applications that involve *in vivo* administration to a human such as, *e.g.*, use as radiation sensitizers for the treatment of neoplastic disease or use in methods to reduce the side effects of, *e.g.*, cancer therapy.

Humanized antibodies can be prepared using a variety of techniques including, for example: (1) grafting the non-human complementarity determining regions (CDRs) onto a  
30 human framework and constant region (a process referred to in the art as "humanizing"), or, alternatively, (2) transplanting the entire non-human variable domains, but "cloaking" them with a human-like surface by replacement of surface residues (a process referred to in the art



as “veneering”). In the present invention, humanized antibodies include both “humanized” and “veneered” antibodies. These methods are disclosed in, *e.g.*, Jones et al., *Nature* 321:522-525 (1986); Morrison et al., *Proc. Natl. Acad. Sci. U.S.A.*, 81:6851-6855 (1984); Morrison and Oi, *Adv. Immunol.*, 44:65-92 (1988); Verhoeyer et al., *Science* 239:1534-1536 (1988); Padlan, *Molec. Immun.* 28:489-498 (1991); Padlan, *Molec. Immunol.* 31(3): 169-217 (1994); and Kettleborough, C.A. et al., *Protein Eng.* 4(7):773-83 (1991) each of which is incorporated herein by reference.

The phrase “complementarity determining region” refers to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin-binding site. *See, e.g.*, Chothia et al., *J. Mol. Biol.* 196:901-917 (1987); Kabat et al., U.S. Dept. of Health and Human Services NIH Publication No. 91-3242 (1991). The phrase “constant region” refers to the portion of the antibody molecule that confers effector functions. In the present invention, mouse constant regions are substituted by human constant regions. The constant regions of the subject-humanized antibodies are derived from human immunoglobulins. The heavy chain constant region can be selected from any of the five isotypes: alpha, delta, epsilon, gamma or mu.

One method of humanizing antibodies comprises aligning the non-human heavy and light chain sequences to human heavy and light chain sequences, selecting and replacing the non-human framework with a human framework based on such alignment, molecular modeling to predict the conformation of the humanized sequence and comparing to the conformation of the parent antibody. This process is followed by repeated back mutation of residues in the CDR region which disturb the structure of the CDRs until the predicted conformation of the humanized sequence model closely approximates the conformation of the non-human CDRs of the parent non-human antibody. Humanized antibodies can be further derivatized to facilitate uptake and clearance, *e.g.* via Ashwell receptors. *See, e.g.*, U.S. Patent Nos. 5,530,101 and 5,585,089 which patents are incorporated herein by reference.

Humanized antibodies to proteins according to the invention can also be produced using transgenic animals that are engineered to contain human immunoglobulin loci. For example, WO 98/24893 discloses transgenic animals having a human Ig locus wherein the animals do not produce functional endogenous immunoglobulins due to the inactivation of endogenous heavy and light chain loci. WO 91/10741 also discloses transgenic non-primate mammalian hosts capable of mounting an immune response to an immunogen, wherein the

antibodies have primate constant and/or variable regions, and wherein the endogenous immunoglobulin-encoding loci are substituted or inactivated. WO 96/30498 discloses the use of the Cre/Lox system to modify the immunoglobulin locus in a mammal, such as to replace all or a portion of the constant or variable region to form a modified antibody molecule. WO 94/02602 discloses non-human mammalian hosts having inactivated endogenous Ig loci and functional human Ig loci. U.S. Patent No. 5,939,598 discloses methods of making transgenic mice in which the mice lack endogenous heavy chain loci, and express an exogenous immunoglobulin locus comprising one or more xenogeneic constant regions.

Using a transgenic animal described above, an immune response can be produced to a selected antigenic molecule, and antibody-producing cells can be removed from the animal and used to produce hybridomas that secrete human monoclonal antibodies. Immunization protocols, adjuvants, and the like are known in the art, and are used in immunization of, for example, a transgenic mouse as described in WO 96/33735. This publication discloses monoclonal antibodies against a variety of antigenic molecules including IL-6, IL-8, TNF, human CD4, L-selectin, gp39, and tetanus toxin. The monoclonal antibodies can be tested for the ability to inhibit or neutralize the biological activity or physiological effect of the corresponding protein. WO 96/33735 discloses that monoclonal antibodies against IL-8, derived from immune cells of transgenic mice immunized with IL-8, blocked IL-8-induced functions of neutrophils. Human monoclonal antibodies with specificity for the antigen used to immunize transgenic animals are also disclosed in WO 96/34096.

In the present invention, proteins and variants thereof according to the invention are used to immunize a transgenic animal as described above. Monoclonal antibodies are made using methods known in the art, and the specificity of the antibodies is tested using isolated colon or colorectal proteins according to the invention.

Methods for preparation of the human or primate protein according to the invention or an epitope thereof include, but are not limited to chemical synthesis, recombinant DNA techniques or isolation from biological samples. Chemical synthesis of a peptide can be performed, for example, by the classical Merrifield method of solid phase peptide synthesis (Merrifield, *J. Am. Chem. Soc.* 85:2149, 1963 which is incorporated by reference) or the FMOC strategy on a Rapid Automated Multiple Peptide Synthesis system [E. I. du Pont de Nemours Company, Wilmington, DE) (Caprino and Han, *J. Org. Chem.* 37:3404 (1972) which is incorporated by reference].

Polyclonal antibodies can be prepared by immunizing rabbits or other animals by injecting antigen followed by subsequent boosts at appropriate intervals. The animals are bled and sera assayed against purified protein usually by ELISA or by bioassay based upon the ability to block the action of a gene according to the invention. When using avian species, *e.g.*, chicken, turkey and the like, the antibody can be isolated from the yolk of the egg. Monoclonal antibodies can be prepared after the method of Milstein and Kohler by fusing splenocytes from immunized mice with continuously replicating tumor cells such as myeloma or lymphoma cells. [Milstein and Kohler, *Nature* 256:495-497 (1975); Gutfre and Milstein, *Methods in Enzymology: Immunochemical Techniques* 73:1-46, Langone and Banatis eds., Academic Press, (1981) which are incorporated by reference]. The hybridoma cells so formed are then cloned by limiting dilution methods and supernates assayed for antibody production by ELISA, RIA or bioassay.

The unique ability of antibodies to recognize and specifically bind to target proteins provides an approach for treating an overexpression of the protein. Thus, another aspect of the present invention provides for a method for preventing or treating diseases involving overexpression of the a protein according to the invention by treatment of a patient with antibodies to specific tumor antigen according to the invention.

Specific antibodies, either polyclonal or monoclonal, to the protein can be produced by any suitable method known in the art as discussed above. For example, murine or human monoclonal antibodies can be produced by hybridoma technology or, alternatively, the tumor protein, or an immunologically active fragment thereof, or an anti-idiotypic antibody, or fragment thereof can be administered to an animal to elicit the production of antibodies capable of recognizing and binding to the tumor protein. Antibodies can be of any class or subclass, *e.g.*, IgG, IgA, IgM, IgD, and IgE or in the case of avian species, IgY, and subclasses thereof.

The availability of isolated human or primate protein according to the invention allows for the identification of small molecules and low molecular weight compounds that inhibit the binding of the protein to binding partners, through routine application of high-throughput screening methods (HTS). HTS methods generally refer to technologies that permit the rapid assaying of lead compounds for therapeutic potential. HTS techniques employ robotic handling of test materials, detection of positive signals, and interpretation of data. Lead compounds can be identified via the incorporation of radioactivity or through

optical assays that rely on absorbance, fluorescence or luminescence as read-outs. [Gonzalez, J.E. *et al.*, *Curr. Opin. Biotech.* 9:624-631 (1998)].

Model systems are available that can be adapted for use in high throughput screening for compounds that inhibit the interaction of a protein with its ligand, for example by competing with the protein for ligand binding. Sarubbi *et al.*, *Anal. Biochem.* 237:70-75 (1996) describe cell-free, non-isotopic assays for discovering molecules that compete with natural ligands for binding to the active site of IL-1 receptor. Martens, C. *et al.*, *Anal. Biochem.* 273:20-31 (1999) describe a generic particle-based nonradioactive method in which a labeled ligand binds to its receptor immobilized on a particle; label on the particle decreases in the presence of a molecule that competes with the labeled ligand for receptor binding.

The therapeutic gene polynucleotides and polypeptides of the present invention can be utilized in gene delivery vehicles. The gene delivery vehicle can be of viral or non-viral origin (*see generally*, Jolly, *Cancer Gene Therapy* 1:51-64 (1994); Kimura, *Human Gene Therapy* 5:845-852 (1994); Connelly, *Human Gene Therapy* 1:185-193 (1995); and Kaplitt, *Nature Genetics* 6:148-153 (1994)). Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic according to the invention can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches. Expression of such coding sequences can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence can be either constitutive or regulated.

The present invention can employ recombinant retroviruses which are constructed to carry or express a selected nucleic acid molecule of interest. Retrovirus vectors that can be employed include those described in EP 0 415 731; WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; U.S. Patent No. 5,219,740; WO 93/11230; WO 93/10218; Vile and Hart, *Cancer Res.* 53:3860-3864 (1993); Vile and Hart, *Cancer Res.* 53:962-967 (1993); Ram *et al.*, *Cancer Res.* 53:83-88 (1993); Takamiya *et al.*, *J. Neurosci. Res.* 33:493-503 (1992); Baba *et al.*, *J. Neurosurg.* 79:729-735 (1993); U.S. Patent No. 4,777,127; GB Patent No. 2,200,651; and EP 0 345 242. Recombinant retroviruses useful in accordance with the present invention include those described in WO 91/02805.

Packaging cell lines suitable for use with the above-described retroviral vector constructs can be readily prepared (*see* PCT publications WO 95/3 0763 and WO 92/05266), and used to create producer cell lines (also termed vector cell lines) for the production of

recombinant vector particles. For example, packaging cell lines can be prepared from human (such as HT1080 cells) or mink parent cell lines, thereby allowing production of recombinant retroviruses that can survive inactivation in human serum.

The present invention also employs alphavirus-based vectors that can function as gene delivery vehicles. Vectors can be constructed from a wide variety of alphaviruses, including, for example, Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532). Representative examples of such vector systems include those described in U.S. Patent Nos. 5,091,309; 5,217,879; and 5,185,440; and PCT Publication Nos. WO 92/10578; WO 94/21792; WO 95/27069; WO 95/27044; and WO 95/07994.

Gene delivery vehicles of the present invention can also employ parvovirus such as adeno-associated virus (AAV) vectors. Representative examples include the AAV vectors disclosed by Srivastava in WO 93/09239, Samulski et al., *J. Vir.* 63: 3822-3828 (1989); Mendelson et al., *Virol.* 166: 154-165 (1988); and Flotte et al., *P.N.A.S.* 90: 10613-10617 (1993).

Representative examples of adenoviral vectors include those described by Berkner, *Biotechniques* 6:616-627 (Biotechniques); Rosenfeld et al., *Science* 252:431-434 (1991); WO 93/19191; Kolls et al., *P.N.A.S.* 215-219 (1994); Kass-Bisler et al., *P.N.A.S.* 90: 11498-11502 (1993); Guzman et al., *Circulation* 88: 2838-2848 (1993); Guzman et al., *Cir. Res.* 73: 1202-1207 (1993); Zabner et al., *Cell* 75: 207-216 (1993); Li et al., *Hum. Gene Ther.* 4: 403-409 (1993); Cailaud et al., *Eur. J. Neurosci.* 5: 1287-1291 (1993); Vincent et al., *Nat. Genet.* 5: 130-134 (1993); Jaffe et al., *Nat. Genet.* 1: 372-378 (1992); and Levrero et al., *Gene* 101: 195-202 (1992). Exemplary adenoviral gene therapy vectors employable in this invention also include those described in WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655. Administration of DNA linked to kill adenovirus as described in Curiel, *Hum. Gene Ther.* 3: 147-154 (1992) can be employed.

Other gene delivery vehicles and methods can be employed, including polycationic condensed DNA linked or unlinked to kill adenovirus alone, for example Curiel, *Hum. Gene Ther.* 3: 147-154 (1992); ligand-linked DNA, for example see Wu, *J. Biol. Chem.* 264: 16985-16987 (1989); eukaryotic cell delivery vehicles cells, for example see U.S. Serial No. 08/240,030, filed May 9, 1994, and U.S. Serial No. 08/404,796; deposition of

photopolymerized hydrogel materials; hand-held gene transfer particle gun, as described in U.S. Patent No. 5,149,655; ionizing radiation as described in U.S. Patent No. 5,206,152 and in WO 92/11033; nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip, *Mol. Cell Biol.* 14:2411-2418 (1994), and in Woffendin, *Proc. Natl. Acad. Sci.* 91:1581-1585 (1994).

Naked DNA can also be administered directly to a subject. Exemplary naked DNA introduction methods are described in WO 90/11092 and U.S. Patent No. 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm. Liposomes that can act as gene delivery vehicles are described in U.S. Patent No. 5,422,120, PCT Patent Publication Nos. WO 95/13 796, WO 94/23697, and WO 9 1/14445, and EP No. 0 524 968.

Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin et al., *Proc. Natl. Acad. Sci. USA* 91(24): 11581-11585 (1994). Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in U.S. Patent No. 5,149,655; use of ionizing radiation for activating transferred gene, as described in U.S. Patent No. 5,206,152 and PCT Patent Publication No. WO 92/11033.

### EXAMPLES

The following Examples have been included to illustrate modes of the invention. Certain aspects of the following Examples are described in terms of techniques and procedures found or contemplated by the present co-inventors to work well in the practice of the invention. These Examples illustrate standard laboratory practices of the co-inventors. In light of the present disclosure and the general level of skill in the art, those of skill will appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the invention.

Example 1Identification of CICO1-CICO3 Genes

Through a collaboration with Analytical Pathology Medical Group (at Grossmont Hospital), IDEC obtained pairs of snap frozen normal and malignant colon tissue removed during surgery. RNA was extracted from 10 pairs of those samples and submitted for GeneTag analysis at Celera/Applied Bio Systems (ABI). In brief, the RNA was reverse transcribed into cDNA, digested with a restriction enzyme, and linkers were ligated to the cDNA library. The library was amplified using the linker sequences as a primer with an additional nucleotide (A, T, G, or C) (+1 PCR) to generate 16 libraries. The libraries were further amplified using the linker sequences as primers with an additional two nucleotides (+2 PCR) to generate 256 libraries. Fluorescently labeled products from these +2 PCR reactions were separated by capillary electrophoresis and the amplified sequences were quantitated. The expression profile obtained from malignant colon RNA was compared to that obtained using RNA from the normal colon. Several sequences were identified to be at least five-fold overexpressed in three of three tumors. The expression results are summarized in Figure 1. Overexpressed sequences were purified and amplified by PCR using the linkers with three additional nucleotides (+3 PCR). The +3 peaks were purified and sequenced. These sequences are set forth below:

20 CICO1 (Celera IDEC Colon Overexpressed 1)(bs213ms134-185)

Using 185 bases of +3 PCR sequence from GeneTag bs213ms134, human tentative human consensus sequence (THC) 684921 was identified from the BLAST database.

bs213ms143-185 Nucleotide Sequence

25 GATCCAGGAGAGGAAGGAGTTTCAGAAGGCAGGAGCTGGTCCTCTATGTCATGAAATGTAGA  
GGGTGAGGCCAAGGAGGACCTGAGAGAAGGTAATTAGATTGTTGTTACAGGCTGGTCCCT  
GTGGCCAGCCACCCACCCACTTTA (SEQ ID NO:1)

THC 684921 Nucleotide Sequence

30 TGAGGAACTGTGGCTTAGAGGAAAAGGTCATTAGTTCATTTTGGGATTT  
GTTGATTTTCAGATGTTTGAGATGTTGAGGATGGATTGTCCAGCAGGCTA

TTAAGATGTGGTGAAGGCTAGAAATGTTGATTTAGGAGGTATTGCCTTCG  
 AGAAGATAAAGGAGGAGAAGAGGAGAGCATCATGCAAGCTAGAGAAGAGA  
 AAGAAGAAAAGTATTCTGGGGAATGTCTCCTTTGGGAGCAGAAAGAAGAC  
 TCTGACGGAGCAGCCATCCAGGAAGTGAATGAGATCCAGGAGAGGAAGG  
 5 AGTTTCAGAAGGCAGGAGCTGGTCCTCTATGTCATGAAATGTAGAGGGTG  
 AGGCCAAGGAGGACCTGAGAGAAGGTAATTAGATTTGGTGTTTACAGGCT  
 GGTCCCTGTGGCCAGCCACCCACCCACTTTAAAATATTTACTCTACAAA  
 TGTTAATGTGTGAAGAGTTGCATGCCAGAATATTTATGGCATCAGTGTTG  
 GTGGATACAGAACATTGGGAAACAACCCATTAATAGCAGAATGGTAAATC  
 10 TGGCCAGTGAATAGTATAGCTTTTTTAAAGGAGGCTGATGTCTGAATTCA  
 CTTTCAAAGTTGTTTACAATGTATTGCTAAAATACAAAATGTTGCAGAA  
 CCATATGTATGAGAGAAACCCCTTTTTCT (SEQ ID NO:2)

CICO 2 (bs222ms233-191)

15 191 bases of the +3 PCR sequence from GeneTag bs222ms233-191 overlapped with the  
 3'UTR of four different hypothetical proteins in the BLAST database.

bs222ms233-191 Nucleotide Sequence

gatcccatggtatgcttgaatctgctccctgaacttcctgccagtgcctccccgtacccca  
 20 aaacaatgtcaccatgggtaccacctaccagaagactgttccctcctcccaagacccttgt  
 ctgcagtggtgctcctgcaggtgcccgtta (SEQ ID NO:3)

chr1\_70\_2399.c mRNA Sequence (coding sequence in CAPITALS, no ATG at start)

AGTGTGGTGATGGTTGTCTTCGACAATGAGAAGGTCCCAGTAGAGCAGCT  
 25 GCGCTTCTGGAAGCACTGGCATTCCCGGCAACCCACTGCCAAGCAGCGGG  
 TCATTGACGTGGCTGACTGCAAAGAAAACCTTCAACACTGTGGAGCACATT  
 GAGGAGGTGGCCTATAATGCACTGTCTTTGTGTGGAACGTGAATGAAGA  
 GGCCAAGGTGTTTCATCGGCGTAACTGTCTGAGCACAGACTTTTCCTCAC  
 AAAAGGGGGTGAAGGGTGTCCCCCTGAACCTGCAGATTGACACCTATGAC  
 30 TGTGGCTTGGGCACTGAGCGCCTGGTACACCGTGCTGTCTGCCAGATCAA  
 GATCTTCTGTGACAAGGGAGCTGAGAGGAAGATGCGCGATGACGAGCGGA  
 AGCAGTTCCGGAGGAAGGTCAAGTGCCCTGACTCCAGCAACAGTGGCGTC



AAGGGCTGCCTGCTGTCGGGCTTCAGGGGCAATGAGACGACCTACCTTCG  
 GCCAGAGACTGACCTGGAGACGCCACCCGTGCTGTTTCATCCCCAATGTGC  
 ACTTCTCCAGCCTGCAGCGGTCTGGAGGGGAGCCCCCTCGGCAGGACCC  
 AGCAGCTCCAACAGGCTGCCTCTGAAGCGTACCTGCTCGCCCTTCACTGA  
 5 GGAGTTTGAGCCTCTGCCCTCCAAGCAGGCCAAGGAAGGCGACCTTCAGA  
 GAGTTCTGCTGTATGTGCGGAGGGAGACTGAGGAGGTGTTTGACGCGCTC  
 ATGTTGAAGACCCCAGACCTGAAGGGGCTGAGGAATGCGATCTCTGAGAA  
 GTATGGGTTCCTGAAGAGAACATTTACAAAGTCTACAAGAAATGCAAGC  
 GAGGAATCTTAGTCAACATGGACAACAACATCATTCAGCATTACAGCAAC  
 10 CACGTCGCCTTCCTGCTGGACATGGGGGAGCTGGACGGCAAAATTCAGAT  
 CATCCTTAAGGAGCTGTAAGgcctctcgagcatccaaaccctcacgacct  
 gcaagggggccagcagggacgtggccccacgccacacacaacctctccaca  
 tgccctcagcgctgttacttgaatgccttccttgagggaagaggcccttga  
 gtcacagacccacagacgtcagggccagggagagacctaggggggtccct  
 15 ggcttgatccccatggtatgcttgaatctgctccctgaacttcctgcca  
 gtgcctccccgtacccccaaaacaatgtcaccatggttaccacctaccag  
 aagactgttcctcctcccaagaccttgtctgcagtggtgctcctgcag  
 gctgcccgttaagatggtggcggcacacgctccctcccgagcaccacgc  
 cagctggtgcgggccccactctctgtcttccttcaacttcagacaaagga  
 20 tttctcaacctttgggtcagttaacttgaaaactcttgattttcagtgcaa  
 atgacttttaaaagacactatatattggagtcctctttctcagacttcctcag  
 cgcaggatgtaaatagcactaacgatcgactggaacaaagtgaccgctgt  
 gtaaaactactgccttgccactcactgttgatacatttcttatttacga  
 ttttcatttggtatatatatataataataactgtatatatatgcaacat  
 25 tttatatattttcatggatatgtttttatcatttcaaaaaatgtgtatttc  
 acatttcttggaacttttttttagctgttattcagtgatgcattttgtatac  
 tcacgtggtatttagtaataaaaaatctatctatgtattacgtcac  
 (SEQ ID NO:4)

30 chr1\_70\_2399.c Amino Acid Sequence  
 SVVMVFDNEKVPVEQLRFWKHWSRQPTAKQRVIDVADCKENFNTVEHI  
 EEVAYNALSFVWNVNEEAKVFIGVNCLSTDFSSQKGVKGVPLNLQIDTYD

CGLGTERLVHRAVCQIKIFCDKGAERKMRDDERKQFRRKVKCPDSSNSGV  
 KGCLLSGFRGNETTYLRPETDLETPPVLFIPNVHFSSLQRSGGAAPSAGP  
 SSSNRLPLKRTCSPFTEEFELPSKQAKEGDLQRVLLYVRRETEEVFDAL  
 MLKTPDLKGLRNAISEKYGFPEENIYKVYKKCKRGILVNMDNNIIQHYSN

5 HVAFLDMGELDGKIQIILKEL (SEQ ID NO:5)

chr1\_70\_2399.f mRNA Sequence (coding sequence in CAPITALS, no ATG at start)

aagttgccccacctctctgagcattggcttccccatctgtgaaagaggag  
 tgctgatgtttgccttctaggggcctagtgaggcttaagggtagcagca  
 10 ggcacacagaaagctagaaatacaggatcactgtgggacggtggggctgg  
 ccacctgggcaggccacttaccagcgccccctctgtctccaggtgttc  
 atcggcgtaaactgtctgagcacagacttttcctcacaaaagggggtgaa  
 ggggtgtccccctgaacctgcagattgacacctatgactgtggcttgggca  
 ctgagcgcttggtacaccgtgctgtctgccagatcaagatcttctgtgac  
 15 aaggagctgagaggaagatgcgcgatgacgagcggaagcagttccggag  
 gaaggtcaagtgccttgactccagcaacagtggcgtcaagggctgcctgc  
 tgtcgggcttcaggggcaatgagacgacctaccttcggccagagactgac  
 ctggagacgccaccctgctgtttcatccccaatgtgcacttctccagcct  
 gcagcggctctggaggggcagccccctcggcaggaccagcagctccaaca  
 20 ggctgcctctgaagcgtacctgctcgcccttcactgaggagtttgagcct  
 ctgccctccaagcaggccaaggaaggcgaccttcagagagttctgctgta  
 tgtgcggaggagactgaggaggtgtttgacgcgctcatgttgaagacc  
 cagacctgaaggggctgaggaatgcgatctctgagaagtatgggttcct  
 gaaGAGAACATTTACAAAGTCTACAAGAAATGCAAGCGAGGAATCTTAGT  
 25 CAACATGGACAACAACATCATTACAGCATTACAGCAACCACGTCGCCTTCC  
 TGCTGGACATGGGGGAGCTGGACGGCAAAATTCAGATCATCCTTAAGGAG  
 CTGTAAggcctctcgagcatccaaaccctcacgacctgcaaggggcccagc  
 agggacgtggccccacgccacacacaacctctccacatgcctcagcgctg  
 ttacttgaatgccttcctgaggggaagaggcccttgagtacagacccac  
 30 agacgtcagggccagggagagacctaggggggtcccctggcctggatcccc  
 atgggtatgcttgaatctgctccctgaacttcctgccagtgctccccgta  
 ccccaaaacaatgtcaccatgggtaccacctaccagaagactgttcct

cctcccaagacccttgtctgcagtggtgctcctgcaggctgcccggttaag  
 atggtggcggcacacgctccctcccgagcaccacgccagctggtgcggc  
 cccactctctgtcttccttcaacttcagacaaaggatttctcaaccttt  
 ggtcagttaacttgaaaactcttgatttttcagtgcaaatacttttataaa  
 5 gacactatattggagtctctttctcagacttcctcagcgcaggatgtaaa  
 tagcactaacgatcgactggaacaaagtgaccgctgtgtaaaactactgc  
 cttgccactcactgttgatacatttcttattttacgattttcatttgta  
 tatatatataaataactgtatatatatatgcaacattttatatattttca  
 tggatatgtttttatcattttcaaaaaatgtgtattttcacattttcttgga  
 10 ttttttttagctgttatttcagtgatgcattttgtatactcacgtggtattt  
 agtaataaaaatctatctatgtattacgtcac (SEQ ID NO:6)

## chr1\_70\_2399.f Amino Acid Sequence

MRDDERKQFRRKVKCPDSSNSGVKGCLLSGFRGNETTYLRPETDLETPPV  
 15 LFIPNVHFSSLQRSGGAAPSAGPSSSNRLPLKRTCSPFTEEFELPSKQA  
 KEGDLQRVLLYVRRETEEVFDALMLKTPDLKGLRNAISEKYGFPEENIYK  
 VYKCKRGILVNMDNNIIQHYSNHVAFLLDMGELDGKIQIILKEL (SEQ ID NO:7)

## C1000572 mRNA Sequence (coding)

20 ATGAAAAGGTCTGTGCGGCTGCTAAAGAACGACCCAGTCAACTTGCAGAA  
 ATTCTCTTACACTAGTGAGGATGAGGCCTGGAAGACGTACCTAGAAAACC  
 CGTTGACAGCTGCCACAAAGGCCATGATGAGAGTCAATGGAGATGATGAG  
 AGTGTTGCGGCCTTGAGCTTCTCTATGATTACTACATGTCGATGCTCTT  
 CCCAGATATCCTGAAAACCTCCCCGGAACCCCCATGTCCAGAGGACTACC  
 25 CCAGCCTCAAAAGTGACTTTGAATACACCCTGGGCTCCCCCAAAGCCATC  
 CACATCAAGTCAGGCGAGTCACCCATGGCCTACCTCAACAAAGGCCAGTT  
 CTACCCCGTCACCCTGCGGACCCCAGCAGGTGGCAAAGGCCTTGCCTTGT  
 CCTCCAACAAAGTCAAGAGTGTGGTGATGGTTGTCTTCGACAATGAGAAG  
 GTCCAGTAGAGCAGCTGCGCTTCTGGAAGCACTGGCATTCCCGGCAACC  
 30 CACTGCCAAGCAGCGGGTCATTGACGTGGCTGACTGCAAAGAAAACCTTCA  
 AACTGTGGAGCACATTGAGGAGGTGGCCTATAATGCACTGTCCTTTGTG  
 TGAACGTGAATGAAGAGGCCAAGGTGTTTCATCGGCGTAAACTGTCTGAG

CACAGACTTTTCCTCACAAAAGGGGTGAAGGGTGTCCCCCTGAACCTGC  
 AGATTGACACCTATGACTGTGGCTTGGGCACTGAGCGCCTGGTACACCGT  
 GCTGTCTGCCAGATCAAGATCTTCTGTGACAAGGGAGCTGAGAGGAAGAT  
 GCGCGATGACGAGCGGAAGCAGTTCCGGAGGAAGGTCAAGTGCCCTGACT  
 5 CCAGCAACAGTGGCGTCAAGGGCTGCCTGCTGTCTGGGCTTCAGGGGCAAT  
 GAGACGACCTACCTTCGGCCAGAGACTGACCTGGAGACGCCACCCGTGCT  
 GTTCATCCCCAATGTGCACTTCTCCAGCCTGCAGCGGTCTGGAGGGAGCC  
 TCCAGCAGCCAGGGGCTCCTCTCATTTTCCTGCGTGTGATGGAAAATGTC  
 TTTTTCACCTTCATTGCAGGCAGCCCCCTCGGCAGGACCCAGCAGCTCCAA  
 10 CAGGCTGCCTCTGAAGCGTACCTGCTCGCCCTTCACTGAGGAGTTTGAGC  
 CTCTGCCCTCCAAGCAGGCCAAGGAAGGCGACCTTCAGAGAGTTCTGCTG  
 TATGTGCGGAGGGAGACTGAGGAGGTGTTTGACGCGCTCATGTTGAAGAC  
 CCCAGACCTGAAGGGGCTGAGGAATGCGATCTCTGAGAAGTATGGGTTC  
 CTGAAGAGAACATTTACAAAGTCTACAAGAAATGCAAGCGAGGAATCTTA  
 15 GTCAACATGGACAACAACATCATTACAGCATTACAGCAACCACGTTCGCTT  
 CCTGCTGGACATGGGGGAGCTGGACGGCAAAATTCAGATCATCCTTAAGG  
 AGCTGTAA (SEQ ID NO: 8)

#### C1000572 Amino Acid Sequence

20 MKRSVRLKNDPVNLQKFSYTSDEAWKTYLENPLTAATKAMMRVNGDDE  
 SVAALSFLYDYMSMLFPDILKTSPEPPCPEDYPSLKSDFEYTLGSPKAI  
 HIKSGESPMAYLNKGQFYPTLRTLPAGGKGLALSSNKVKSVMVVFNEK  
 VPVEQLRFWKHWSRQPTAKQRVIDVADCKENFNTVEHIEEVAYNALSFV  
 WNVNEEAKVFIGVNCLSTDFSSQKGVKGVPLNLQIDTYDCGLGTERLVHR  
 25 AVCQIKIFCDKGAERKMRDDERKQFRRKVKCPDSSNSGVKGCLLSGFRGN  
 ETTYLRPETDLETPPVLFIPNVHFSSLQRSGGSLQQPGAPLIFLRVMENV  
 FFTSLQAAPSAGPSSSNRLPLKRTCSPFTEEFELPSKQAKEGDLQRVLL  
 YVRRETEEVFDALMLKTPDLKGLRNAISEKYGFPEENIYKVYKKCKRGIL  
 VNMDNNIIQHYSNHVAFLLDMGELDGKIQIILKEL (SEQ ID NO: 9)

30

#### ctgChr\_1ctg20.176 mRNA Sequence (coding)

ATGGAGGCAGGGGAGAAAAGCGCTCTGGGTGCCTGGAGCCCGCAGCCCTG

GGCAGCCCCGGGCTACCGCAGGGCGCAAGGGATCCTGGGCTGCGGCCGAG  
GGCGCCGGAAGTCGCCGCCGACCGCCTGGGTCTCGCAGGAAAACAGCCGG  
CGCCCGCAGACTGCCCAGCGTCGGGTTTTCTGAAGAGCCCAGCTCCTCA  
CACCTTGGGGCCTGGTGGGATGGGAGACACTGTCCTGGATGAAGCCGCTG  
5 GGAGAGCTGCCGCCTCCTGTATGCTGAGGTCTGTGCGGCTGCTAAAGAAC  
GACCCAGTCAACTTGCAGAAATTCTTTACTAGTGAGGATGAGGCCTG  
GAAGACGTACCTAGAAAACCCGTTGACAGCTGCCACAAAGGCCATGATGA  
GAGTCAATGGAGATGATGAGAGTGTTGCGGCCTTGAGCTTCCTCTATGAT  
TACTACATGGGTCCCAAGGAGAAGCGGATATTGTCCTCCAGCACTGGGGG  
10 CAGGAATGACCAAGGAAAGAGGTACTACCATGGCATGGAATATGAGACGG  
ACCTCACTCCCCTTGAAAGCCCCACACACCTCATGAAATTCCTGACAGAG  
AACGTGTCTGGAACCCAGAGTACCCAGATTTGCTCAAGAAGAATAACCT  
GATGAGCTTGGAGGGGGCCTTGCCCACCCCTGGCAAGGCAGCTCCCCTCC  
CTGCAGGCCCCAGCAAGCTGGAGGCCGGCTCTGTGGACAGCTACCTGTTA  
15 CCCACCACTGATATGTATGATAATGGCTCCCTCAACTCCTTGTTTGAGAG  
CATTTCATGGGGTGCCGCCACACAGCGCTGGCAGCCAGACAGCACCTTCA  
AAGATGACCCACAGGAGTCGATGCTCTTCCAGATATCCTGAAAACCTCC  
CCGGAACCCCCATGTCCAGAGGACTACCCAGCCTCAAAAGTGACTTTGA  
ATACACCCTGGGCTCCCCCAAAGCCATCCACATCAAGTCAGGCGAGTCAC  
20 CCATGGCCTACCTCAACAAAGGCCAGTTCTACCCCGTCACCCTGCGGACC  
CCAGCAGGTGGCAAAGGCCTTGCTTGTCTCCAACAAAGTCAAGAGTGT  
GGTGATGGTTGTCTTCGACAATGAGAAGGTCCCAGTAGAGCAGCTGCGCT  
TCTGGAAGCACTGGCATTCCCGGCAACCCACTGCCAAGCAGCGGGTCATT  
GACGTGGCTGACTGCAAAGAAAACCTTCAACACTGTGGAGCACATTGAGGA  
25 GGTGGCCTATAATGCACTGTCCTTTGTGTGGAACGTGAATGAAGAGGCCA  
AGGTGTTTCATCGGCGTAAACTGTCTGAGCACAGACTTTTCCTCACAAAAG  
GGGGTGAAGGGTGTCCCCCTGAACCTGCAGATTGACACCTATGACTGTGG  
CTTGGGCACTGAGCGCCTGGTACACCGTGCTGTCTGCCAGATCAAGATCT  
TCTGTGACAAGGGAGCTGAGAGGAAGATGCGCGATGACGAGCGGAAGCAG  
30 TTCCGGAGGAAGGTCAAGTGCCCTGACTCCAGCAACAGTGGCGTCAAGGG  
CTGCCTGCTGTGCGGCTTCAGGGGCAATGAGACGACCTACCTTCGGCCAG  
AGACTGACCTGGAGACGCCACCCGTGCTGTTTCATCCCCAATGTGCACTTC

TCCAGCCTGCAGCGGTCTGGAGGGCTCCAACTGCCTAGTTACCGGCCGCA  
GGACCATCTGCAATTCCCAGCCCTTCTGGGCATGCTGGGGCCCAGGCTGC  
CTCTGAAGCGTACCTGCTCGCCCTTCACTGAGGAGTTTGAGCCTCTGCCC  
TCCAAGCAGGCCAAGGAAGGCGACCTTCAGAGAGTTCTGCTGTATGTGCG  
5 GAGGGAGACTGAGGAGGTGTTTGACGCGCTCATGTTGAAGACCCCAGACC  
TGAAGGGGCTGAGGAATGCGATCTCTGAGAAGTATGGGTTCCTGAAGAG  
AACATTTACAAAGTCTACAAGAAATGCAAGCGAGGAATCTTAGTCAACAT  
GGACAACAACATCATTACAGATTACAGCAACCACGTCGCCTTCCTGCTGG  
ACATGGGGGAGCTGGACGGCAAATTCAGATCATCCTTAAGGAGCTGTAA  
10 (SEQ ID NO:10)

ctgChr\_1ctg20.176 Amino Acid Sequence

MEAGEKSALGAWSPQPWAAPGYRRAQGILGCGRGRKSPPTAWVSQENSR  
RPRAAQRRVFLKSPAPHTLGPGMGDTVLDEAAGRAAASCMLRSVRLLN  
15 DPNVLQKFSYTSSEDAWKTYLENPLTAATKAMMRVNGDDESVAALSFLYD  
YYMGPKEKRILSSSTGGRNDQGKRYHYHMEYETDLTPLESPTHLMKFLTE  
NVSGTPEYPDLLKKNLMSLEGALPTPGKAAPLPAGPSKLEAGSVDSYLL  
PTTDMYDNGSLNSLFESIHGVPPTQRWQPDSTFKDDPQESMLFPDILKTS  
PEPPCPEDYPSLKSDFEYTLGSPKAIHIKSGESPMAYLNKGQFYPVTLRT  
20 PAGGKGLALSSNKVKSVMVVFNEKVPVEQLRFWKHWHSRQPTAKQRVI  
DVADCKENFNTVEHIEEVAYNALSFWNVNNEAKVFIGVNCLSTDFSSQK  
GVKGVPLNLQIDTYDCGLGTERLVHRAVCQIKIFCDKGAERKMRDDERKQ  
FRRKVKCPDSSNSGVKGCLLSGFRGNETTYLRPETDLETPPVLFIPNVHF  
SSLQRSGGLQLPSYRPQDHLQFPALLGMLGPRLPLKRTCSPFTEEFELP  
25 SKQAKEGDLQRVLLYVRRETEEVFDALMLKTPDLKGLRNAISEKYGFPEE  
(SEQ ID NO:11)

CICO3 (bs432ms434-222)

The 222 bases of the +3 PCR sequence from GeneTag bs432ms434-222 overlapped with the  
30 3'UTR of two different hypothetical proteins in the BLAST database.

## bs432ms434-222 Nucleotide Sequence

GATCTGCAATCAGAACTATTGAACTTCTCCATTTCAGACCGCCACTCACACCTATGGGAAAAG  
GGTAATGTATCATCGGCTTAGCAACAGGGAATACTATTTCGTATGATGGAAAATGGGGACAAA  
AGGCTTTGGTACATAAAACATTATTCCTTCCTTGGCCTAAAAACTCATCGCCACCTACATTA

5 (SEQ ID NO:12)

## chr19\_53\_399.c mRNA Sequence

tctggagcagctgaaaaacaaggaagtgaaacagccaattcctgccttaa  
ctaattaaccaccttacgacattccaccattatgacgtgttcctgccct  
10 gccccaaactgatcaatcgaccctgtgacattcttctggacaatgagtccc  
atcatctctccaccatgcaccttgtgactccctcctctgctgacaacaga  
taaccacctttaactgtaactttccacagcctaccccagccctataaagc  
tgccccctctcctatctcccttcgctgactctcttttcagactcagcccac  
ttgcacccaagtgaattaacagccttggtgctcacacaaagcctgtttag  
15 gtggtcttctatacggacatgcttgacacttggtgccaaaatctgggcca  
gggggactccttcgtgagaccggccccctgtcctggccctcattccgtga  
agagatccacctgcgacctcgggtcctcagaccagcccaaggaacatctc  
accaatttcaaateggatctcctcggcttagtggtgaagactgatgctg  
cccgatcgctcagaagcccccttgaccatcacagatgccgagcttcggg  
20 taactcttacggtggaggattcccagccatatgaagacaccctagctgga  
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gaccagaccctacccggtcatttatagcacaccaactgccgtccatctgc  
aggaccctctccattgggttcaccattccagaataaagccatgcccatca  
gacagccagcttgatctctcctcttcctcctggaagccacaagattaggc  
25 cgagagccgatcagacaaacaacctacaacccttaagctcctggcagcgc  
ccagccaaggccatgcttccttgcaacactccttccaaatggccatccca  
gcatgcttccaagcaggcttcacccgttcctctggaccctcatctcttaa  
gacctgccgcctataaaaaggattatatcttgagaccctatcctctaaaa  
ttttttccacacccaaaaacaaaaaatctctgggtcaaaagtctaaaacgc  
30 ttaggctggcaaccatcagatccttgcccatggtgtcctcaagcctactc  
tcatgaaatggacaacagtacacgcatatggggccagttccacatatctg  
gcaaccagaccagcatccaggacaacacaaagatctgcaatcagaactat

tgaacttctccattcagaccgccactcacacctatgggaaaagggtaatg  
 tatcatcggcttagcaacaggggaatactattcgtatgatggaaaatgggg  
 acaaaaggctttggtacataaaacattattccttccttggcctaaaaact  
 catcgccacctacattaaagctaatatgcctgattactgttttttagagaa  
 5 cttatttttattagggcagttccaagctcaaaaatacgctaactggcacct  
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 cttttttttaatcttttcctacaacttcataattttataaataaaaagaca  
 10 aaaatgtcaggcctgtgagctgaagcttagccattgtaaccctgtgacc  
 tgcacatatccgtccaggtggcctgcaggagccaagaagtctggagcagc  
 cgaaaaaccacaaagaagtgaacagccagttcctgccttaactaattaa  
 cccaccttacgacattccaccattatgacttgtccaccattatgacttgt  
 tcctgccttgcctcaactgatcaatcaaccctgtgacattcttctcctgg  
 15 acaatgagtcccatcatctctccaccatgcaccttgtgacccctcctct  
 gctgaggataaccacctttaactgtaactttccacgcctacccaagccct  
 ataaagctgcccctctcctatctcccttactgactctcttttcggactc  
 agcccacttgcacccaagtgaattaacagccttggtgctcacacaaagcc  
 tgattgggtgtcttctatacggacacgcgtgacaggaacctcaacccaaa  
 20 ggcagtctgatgaggtgtctaagataaaagtagcggcacaaaggcttttg  
 taaacagaggcgtttcatgtgggttttcctttcctttccttatatgtgaaa  
 aggtgacagaaaagaaatcttcctaaaagagtc (SEQ ID NO:13)

#### chr19\_53\_399.c Amino Acid Sequence

25 MGPVPHIWQPDQHPGQHKDLQSELLNFSIQTATHTYGKRVMYHRLSNREY  
 YSYDGKWGQKALVHKTLFLPWPKNSSPPTLKLICLITVFRELILLGQFQA  
 QKYANWHLVSYIKMHPRPETY (SEQ ID NO:14)

#### chr19\_53\_399.b mRNA Sequence

30 tctggagcagctgaaaaacaaggaagtgaacagccaattcctgccttaa  
 ctaattaaccaccttacgacattccaccattatgacgtgttcctgcct  
 gcccactgatcaatcgaccctgtgacattcttcttgacaatgagtccc



atcatctctccaccatgcaccttgtgactccctcctctgctgacaacaga  
taaccacctttaactgtaactttccacagcctaccccagccctataaagc  
tgccctctcctatctcccttcgctgactctcttttcagactcagcccac  
ttgcacccaagtgaattaacagccttggtgctcacacaaagcctgtttag  
5 gtggtcttctatacggacatgcttgacacttgggtgccaaaatctgggcca  
gggggactccttcgtgagaccggccccctgtcctggccctcattccgtga  
agagatccacctgcgacctcgggtcctcagaccagcccaaggaacatctc  
accaatttcaaatecggatctcctcggcttagtggctgaagactgatgctg  
cccgatcgcctcagaagcccccttgaccatcacagatgccgagcttcggg  
10 taactcttacggtggaggattcccagccatatgaagacaccctagctgga  
cgatcagtccttgtcaaaagtctgacccctcaaactctacagcctcaatg  
gaccagaccctacccgggtcatttatagcacaccaactgccgtccatctgc  
aggaccctctccattgggttcaccattccagaataaagccatgcccatca  
gacagccagcttgatctctcctcttcctcctggaagccacaagattaggc  
15 cgagagccgatcagacaaacaacctacaacccttaagctcctggcagcgc  
ccagccaaggccatgcttccttgcaacactccttccaaatggccatcca  
gcatgcttccaagcaggcttcatccgttcctctggaccctcatctcttaa  
gacctgccgcctataaaaaggattatatcttgagaccctatcctctaaaa  
ttttttccacaccccaaaaacaaaaaatctctgggtcaaaagtctaaaacgc  
20 ttaggctggcaaccatcagatccttgcccatgggtgtcctcaagcctactc  
tcatgaaatggacaacagtacacgcatatggggccagttccacatatctg  
gcaaccagaccagcatccaggacaacacaaaagtatgttggttggttag  
agggttgggacatttcactctttgccagcctcagcttaatccaggagac  
aaagattatcttcttattatctcttctgcataggatctgcaatcagaac  
25 tattgaacttctccattcagaccgccactcacacctatgggaaaagggt  
atgtatcatcggttagcaacagggaataactattcgtatgatggaaaatg  
gggacaaaaggctttggtacataaaacattattccttccttggcctaaaa  
actcatcgccacctacattaaagctaatatgcctgattactgtttttaga  
gaacttatcttattagggcagttccaagctcaaaaatacgctaactggca  
30 ccttggttagctacataaaaatgcaccctagacccgaaacttactagactc  
attataaaattttctttaagggtgtccacgcagtccttgggtcacacttgaa  
gcagtcgggagaaatatcagccctaccccagtaatcccagaaggaactt

acacttttttttaatcttttcctacaacttcataattttataaataaaaag  
 acaaaaatgtcaggcctgtgagctgaagcttagccattgtaaccctgtg  
 acctgcacatatccgtccaggtggcctgcaggagccaagaagtctggagc  
 agccgaaaaaccacaaagaagtgaacagccagttcctgccttaactaat  
 5 taaccaccttacgacattccaccattatgacttgccaccattatgact  
 tgttcctgcctgccccaaactgatcaatcaaccctgtgacattcttctcc  
 tggacaatgagtcctcatctctccaccatgcacctgtgacccccctcc  
 tctgctgaggataaccacctttaactgtaactttccacgcctaccaagc  
 cctataaagctgccccctctcctatctcccttcactgactctcttttcgga  
 10 ctcagcccacttgacccaagtgaattaacagccttggtgctcacacaaa  
 gcctgattgggtgtcttctatacggacacgcgtgacaggaaacctcaacc  
 aaaggcagtctgatgaggtgtctaagataaaagtagcggcacaaaggctt  
 ttgtaaacagaggcgtttcatgtggttttcctttcctttccttatatgtg  
 aaaaggtgacagaaaagaaatcttcctaaaagagtc (SEQ ID NO:15)

15

chr19\_53\_399.b Amino Acid Sequence

CCPIASEAPWTITDAELRVTLTVEDSQPYEDTLAGRSVLVKS LTPQTLQP  
 QWTRPYPVIYSTPTAVHLQDPLHWVHHSRIKPCPSDSLSDLSSSSWKPQD  
 (SEQ ID NO:16)

20

## EXAMPLE 2

### Identification of Candidate Genes 1-4

Four DNA sequences were identified as being overexpressed in colon carcinoma using the  
 Gene Logic (Gaithersburg, Maryland) Gene Express Oncology Datasuite. The sequences  
 25 were identified in a datasuite search, which compared gene expression in colon tumors with  
 expression in normal tissues. These sequences represent genes and encode antigens which  
 are targets for colon cancer therapeutics.

The nucleotide sequences of each candidate gene are listed below. The first sequence listed  
 30 for each candidate gene was obtained directly from the public NCBI database  
 ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) and corresponds to the GENBANK Accession No. number listed in

the Gene Logic database. Additional sequence information was obtained by sequencing EST clones corresponding to each candidate gene.

Candidate 1: GENBANK Accession No. W91975

5 W91975/IMAGE Clone 415310 3' mRNA Sequence

GGCTTCTAAGGTACATTATGTTTTACTTTAATAAATAAAAATTAACCTT  
 GAAGAAAAATGCAGNGCCCTATTTAATTGCTCTGCATGAAATGTACAG  
 AAACGGCAACCTCTGCGATTCTAAGCACTGTGAACGCCCCAGCCACAC  
 CGTGTCAACAAACCGTGTGGCACTTGGGAGAAGGCAGGGGTGATTTAC  
 10 GANTAGTCATGTTTCGCCTCCACCCGAGTCACTGCCAAGGAGTGGACA  
 GTGACACTGAATAAGCATNCGGNGCACCTCCTTCGGGAAGGGACTTGG  
 CTGACATGGTAGGCCTTCCCCTGAGCCTGTACTTTGTCTTGCTGGG  
 CAGCACTCCANTCATGGGAAGGAACAATGANCAAGGCGTGGTGGTGGG  
 GGTGNGTAGGCCTGAGCGCCGTTTTCCATGGTGACCTTCACTGAGCAG  
 15 GCAGCAGGCACTGATGGGCAGTTGAGNCTGGNAGGAGTCAGGTCCTGG  
 TCNTGCCTCTGGTGTAACGCAGCANGCCATCAAAGGT (SEQ ID NO:17)

IMAGE Clone 194681 T3 & T7 Consensus Sequence

AGAATTCGGCAGGAGNTTTTTTTTCTCTTAGATCTCCAGGTTCCCTTACCCCGGGA  
 20 AGCCTTTCTTCATCCCACCGTCCTGGGGCGTTNCACAGTGCTTAGAATCGCAGAGGTTGC  
 CGTTTCTGTACATTTTCATGCAGAGCAATTAAATAGGGCACTGCATTTTTCTTCAAGTTAA  
 TTTTTATTTATTAAAGTAAACATAATGTACCTTAGAAGCCAGACAGTCCTACAAGCTTA  
 TTATGTTGTACAGCGGCGTTCCGTCCCCCTCCCCAGCCCTCTCTTTCTAGAGGCAGCCAA  
 TTTCAGCTGTCTCTCTCTGCTTACCTACATATTTCCATGTTTCTTGTTTCATCACCTGGT  
 25 GGCACCTTCAGTCTGGAAACACCTGCCCTTCACTTTAGGGGAATTGGGCCCCTGTTCGTT  
 TGATAAGTTTTCTTACCATTTTCTGATTTGTTTTTCTTTCTGGAAAATGTATTAGTCAG  
 ATGTAGGCTTTTCTGGATTAATCCTTCAACTTTCCTTTCTTTCCCTTCCTGCCTGT  
 CTCCCTGTTCTTTCTTACACTTTCTCAGGGAGATTCTTGACTGTATTTTCCAACCTTTGTA  
 TCGACCATTTTACTTTTCTGCCATATTTTCAATGTTTACTGATGTTTCTCTGCCCTTTC  
 30 AGTGCATCCTGGTTTTATTTTCATGTTAGACTGAATCCATGTGAAATTGATAACAGGTTTT  
 CAGCCCACACACACACACACAAAAA (SEQ ID NO:18)

Candidate 2: GENBANK Accession No. AI694242

## AI694242/IMAGE Clone 2327838 3' mRNA Sequence

TTTTGTTGGCTGAGGCGGTATTTTCCTTTTATTGCTGTTATGAGATT  
CAACATTTTTTCCAGAAATAACTTCTGAAAAGTGTGCCTAGATTTTG  
5 AACACTTGTGATCCTAACATGTGGTGAGAAAGGCTTTTCAAACACA  
CACGTGTGGACAGAGGTCCACACACGGATACGTGTGCACACACGGGT  
GCCTTGGGCGTGCCTCTTCCAAAAGGGGCGAGTACAGCTATCAACTT  
GTGACTTCCAGGAGGCCTGGGTTTGCCTACGAAGGGGCCGTGTTCCC  
AGTTGGCGTTTACACGTGGTGTACACACACAGGCACAGGCACCGTGT  
10 CCCAAGGCCATCTCCCAAGGGCACCCGCAGACACTGGGCAGCCTTCT  
CCGAAGCTGTCAGTGTCTTCTCGTGAGAGGATGATGAAGAGGATG  
TGTTTCCGCCGCCTCATCCACAGGCCGGCTG (SEQ ID NO:19)

## IMAGE Clone 2327838 T3 &amp; T7 Consensus Sequence

15 NAAAANGGCGCCNGNCCCANNTAAAATNNACCCNCCTAAAGGGGAAAAACTNNGGCGGCC  
GCCTTCGTTTTTTTTTTTTTTTTTTTGTGGTGGCTGAGGCGGTATTTTCCTTTTATTGCT  
GTTAAGAGATTCAACATTTTTTCCAGAAATAACTTCTGAAAAGGGGGCCTNAGATTTTGA  
ACACTTGGGATCCTAACAGGGGGTGAGAAAGGCTTTTCAAACACACNACGGGTGGACAG  
AGGTCCACACACGGNATACGGGGGCACACACGGGTGCCTTGGGCGTGCGTCTTCCAAAAG  
20 GGGCGAGNTACAGCTATCAACTTGTGACTTCCAGGAGGCCTGGGTTTGCCTACGAAGGGG  
CCGNTGTTCCAGTTGGCGTTCACACGTGGTGTACACACACAGGCACAGGCACCNCTGTC  
CCAANGGCCATCTNCCCAAGGGCACCCGCAGACACTGGGCAGCCTTCTCCGAAGCTGTCA  
GTGTCCTTCTCGTGAGAGGATGATGAAGAGGATGTGGTTTCCGCCGCCTCATCCACAGG  
CCGGCTGCCCACGGAGCCTTAGACATCGAGGCCAGAGCGACAGAAGCCTGTGTGCTGACC  
25 GGCCTGGTCTCCTTTGACGTCTCGAGCAGCTTGGCAGGGTGGGAAAAGTAGCCTGAGAGT  
GATCCCCGGGCAGTGTCCGAGGCTCTGCCGTCCCCACCCCCACAGGCATCCAGGGGAGAG  
AAACAACCTGCGCCTGCGAGGCCGTGCGGACCCCGCTCCACTCACCCCGCCTGGGGGGCC  
AGAACCACCTCCAGGGGCTTCCGCCAGTGCCGCAGTTGCTGACCCAGGCAAACCTCGC  
CGCTCCTGCCCCGGCGGGCCTGGGATTTGCGAATGTGTGAAGGCATTAGCTGCCAGTTG  
30 TAACTGGAACCCAGCCTAGAGGCCTCACTCCTCCAGCAGGAAGCCTTGTAATGCAGCGAA  
TCTGAACCCGGCCCAGCGTCCAGAGACAGGAAGCATTAATAGGAGCGAATGTGAACACTG  
TTCGCGCCCTGGCTGCGATTTATTGCCGATTGTGGGGAAAACATCAGTTGGTTGCAGAGT

TTCATTTCATCTTTAGGGACAGGACCGGTGTGTCTGGGTGGCAGTTTAGAGAGCTGGGACA  
GTCGGCATCACTCTGGGTGGCTCCTCTCAANCCCTGGTGCCTCGTGCCGAATTCTGGCCT  
CGAGGCATTCTNAGGGGCTNTATNC (SEQ ID NO:20)

5 Candidate 3: GENBANK Accession No. AI680111

AI680111/IMAGE Clone 2252029 3' mRNA Sequence

TTTTTTTTTTTGTGGATAAATATATTAGCAAATGAATATATTTCTTAACATAGTGCCT  
GATTCAAGCGTCTGTCTGGTTCAAATATAAATACCCATGTGGGTACCTAGGTGCTAGTC  
TCCCCACTAACTGAGGGAAAAAGGTTCCCAGGTGGGGTCCTCTGCCCACCTTGCCACCA  
10 CATTCACATTCCAAATGGGATAATGCCTGAGGGGCCATGAGTGGTCAGGCTGCCCTGGG  
GTGAATGTCACCCTGATGAGGCCCATCAGCTCTTGTCCACTCAGTGAGGCCAGACTTGT  
GCTCTAATCCACT (SEQ ID NO:21)

IMAGE Clone 2324560 T7 Sequence

15 CTNTGTANAAAGCTGGGTACGCGTAAGCTTGGGCCCCCTCGAGGGATACTCTAGAGCGGC  
CGCCCTTTTTTTTTTTTTTTTTTGTGGATAAATATATTAGCAAATAAATATATTTCTTAACA  
TAGTGCCTGATTCAAGCGTCTGTCTGGTTCAGATATAAATACCCATGTGGGTACCTAGG  
TGCTAGTCTCCCCACTAACTGAGGGAAAAAGGTTCCCAGGTGGGGTCCTCTGCCCACCTT  
TGCCACCACATTACATTCCAAATGGGATAATGCCTGAGGGGCCAAGAGTGGTCAGGCT  
20 GCCCTGGGGTGAATGTCACCCTGATGAGGCCCATCAGCTCTTGTCCACTCAGTGAGGCC  
AGACTTGTGCTCTAATCCACTCTCCTGTGGGTCCCTGGCCTGTATGGCTTATACTGGGG  
AGCTGGGCCTCTGGGCTGTCCAAACCCAAGGGTCACACTTTGCTTTTCCTTTGTTGTCC  
CCATTTTCCATCCTTGCTCTAAGACAAAACCTTTCCCAGAGAAGAACTCTTTGTTGTCC  
CCGCTCAGCTGTAATTCTGCCTTTTCTACCTTCATTCCATCCTTCCTCTGCCCAGATAA  
25 AGTCCAGCAGAAATCCTCCTTTCTACCTCTCTGGGACTCTGAGACAGGAAATCTTCAA  
GGAGGAGTTTTTCCCTCCCCACTATTCTTATTCTCAACCCCCAGAAGAACCAANGGCTG  
CTGTACCCCCCTCAGGGACAGAACTCCACACTATANGGGGGAAAGNTTCANGGGACCCC  
TTCCTTTTANTGCTCANGGCTCCACCTATGCTACTGGNTCCTTTTGGCAAAAAGGNAA  
ATGANAGAGCCAGGGGTGCCCCNTGATGTAACANCCNTTACTGGGGANGGGNCCAANG  
30 NNGGTGNTCAAAGNNCCCCNAGGAGGGAGGNGANAAGGGGTCATGNGTTCTGCTNAANC  
CNCTGGTTGGTATAAANTTGANGNTTGGGGTGANGGAAACCAAAAANGGNTGGAAAAAG  
NAAAACACCTTTNNAACCCCTGGGTACCNANATAAGNTTTTGGCCCNAAAAANTCNGC

CNNCAAGGGATCCGCCCCNCCCCCCCAGGGAAAAANTTGGTTCCTNNGGNGAAAAGGAN  
TTTNCCCCCNCAAATTTTNCCNAAAAGNTTTGGAANTTGNAANAANAAGGANCTT  
CCCCCCCCCNCCACAAAAA (SEQ ID NO:22)

5 IMAGE Clone 2324560 SP6 Sequence

CNNTTNCAAAAAGCAGGCTGGTACCGGTCCGGAATTCGCGGGATATCGTCGACCCACGC  
CGTCCGGTTTGCTGGTGTGCTGAAATAACTCCAGCAGAAGGAAAATTAATGCAGTCCC  
ACCCGCTGTACCTGTGCAATGCCAGTGATGACGACAATCTGGAGCCTGGATTCATCAGC  
ATCGTCAAGCTGGAGAGTCCTCGACGGGCCCCCGCCCTGCCTGTCACTGGCTAGCAA  
10 GGCTCGGATGGCGGGTGAGCGAGGAGCCAGTGCTGTCTCTTTGACATCACTGAGGATC  
GAGCTGCTGCTGAGCAGCTGCAGCAGCCGCTGGGGCTGACCTGGCCAGTGGTGTGATC  
TGGGGTAATGACGCTGAGAAGCTGATGGAGTTTGTGTACAAGAACCAAAAGGCCCATGT  
GAGGATTGAGCTGAAGGAGCCCCCGCCTGGCCAGATTATGATGTGTGGATCCTAATGA  
CAGTGGTGGGCACCATCTTTGTGATCATCCTGGCTTCGGTGCTGCGCATCCGGTGCCGC  
15 CCCC GCCACAGCAGGCCGGATCCGCTTCAGCAGAGAACAGCCTGGGCCATCAGCCAGCT  
GGCCACCAGGAGGTACCAGGCCAGCTGCAGGCAGGCCCGGGGTGAGTGGCCAGACTCAG  
GGAGCAGCTGCAGCTCAGCCCCTGTGTGTGCCATCTGTCTGGAGGGAGTTCTCTGAGGG  
GGCAGGAGCTACGGGTCAATTCCTGCCTCCATGAGTTCCATCGTAACTGTGTGGACCC  
CTGGNTACATCAGCATCCGGACTTGCCCCCTCTTGATGGTTCAACATCACANAGGGGA  
20 GATCCNTTTTCCNGTCCCTGGGAACCTCTNCNATCTTACCAAGAACCAGGGTCGGAAG  
ACTCCCCCTCATTTCNCCAGCATCCCCGGCATGNCCCACTACACCNTCCCTGGTNGCC  
TACCTGTTNGGGCCCTTCCCCGGAATGCAGGGGNTNNGGCCCCCNCAACTGGGTCCTT  
TCCTGCCNTCCAGGNAGCCAGGCATGGGCCCCCGAATCACCCCTTCCCNAANATGGA  
NNATCCCCCGGGTTCCAGGAAAACAAACAACCNCTGGAAGGAANCCNNNACCCNTNNC  
25 CCNAAGGCTGGGGAANGNAACNCCCCNATTCCCNNTNNANGANCCCTNNGTTTNCNCN  
AGGCCCCTNACCCGGGCCNNGCCCCNAAACAAAGGGANTTGANAAANT (SEQ ID  
NO:23)

These sequences correspond to hypothetical gene FLJ20315/GENBANK Accession No.  
30 No. AK000322.

## AK000322 Nucleotide Sequence

AAAAAAAAAAAACTTTAGAGAAAGGAAGGGCCAAACTACGACTTGGCTTTCTGAAACG  
GAAGCATAAATGTTCTTTTCTCCATTTGTCTGGATCTGAGAACCTGCATTGTTGTTATTA  
GCTAGTGGAAGCAGTATGTATGGTTGAAGTGCATTGCTGCAGCTGGTAGCATGAGTGGT  
5 GGCCACCAGCTGCAGCTGGCTGCCCTCTGGCCCTGGCTGCTGATGGCTACCCTGCAGGC  
AGGCTTTGGACGCACAGGACTGGTACTGGCAGCAGCGGTGGAGTCTGAAAGATCAGCAG  
AACAGAAAGCTGTTATCAGAGTGATCCCCTTGAAAATGGACCCACAGGAAAATGAAT  
CTCACTTTGGAAGGTGTGTTTGCTGGTGTGCTGAAATAACTCCAGCAGAAGGAAAATT  
AATGCAGTCCCACCCACTGTACCTGTGCAATGCCAGTGATGACGACAATCTGGAGCCTG  
10 GATTCATCAGCATCGTCAAGCTGGAGAGTCCTCGACGGGCCCCCGCCCCCTGCCTGTCA  
CTGGCTAGCAAGGCTCGGATGGCGGGTGAGCGAGGAGCCAGTGCTGTCCTCTTTGACAT  
CACTGAGGATCGAGCTGCTGCTGAGCAGCTGCAGCAGCCGCTGGGGCTGACCTGGCCAG  
TGGTGTGATCTGGGGTAATGACGCTGAGAAGCTGATGGAGTTTGTGTACAAGAACCAA  
AAGGCCCATGTGAGGATTGAGCTGAAGGAGCCCCCGCCCTGGCCAGATTATGATGTGTG  
15 GATCCTAATGACAGTGGTGGGCACCATCTTTGTGATCATCCTGGCTTCGGTGCTGCGCA  
TCCGGTGCCGCCCCCGCCACAGCAGGCCGGATCCGCTTCAGCAGAGAACAGCCTGGGCC  
ATCAGCCAGCTGGCCACCAGGAGGTACCAGGCCAGCTGCAGGCAGGCCCGGGGTGAGTG  
GCCAGACTCAGGGAGCAGCTGCAGCTCAGCCCCTGTGTGTGCCATCTGTCTGGAGGAGT  
TCTCTGAGGGGCAGGAGCTACGGGTCAATTCCTGCCTCCATGAGTTCATCGTAACTGT  
20 GTGGACCCCTGGTTACATCAGCATCGGACTTGCCCCCTCTGCGTGTTCAACATCACAGA  
GGGAGATTCATTTTCCCAGTCCCTGGGACCCCTCTCGATCTTACCAAGAACCAGGTGAA  
GACTCCACCTCATTCGCCAGCATCCCGGCCATGCCCACTACCACCTCCCTGCTGCCTAC  
CTGTTGGGCCCTTCCCGGAGTGCACTGGCTCGGCCCCACGACCTGGTCCCTTCCTGCC  
ATCCCAGGAGCCAGGCATGGGCCCTCGGCATCACCGCTTCCCCAGAGCTGCACATCCCC  
25 GGGCTCCAGGAGAGCAGCAGCGCCTGGCAGGAGCCAGCACCCCTATGCACAAGGCTGG  
GGAATGAGCCACCTCCAATCCACCTCACAGCACCTGCTGCTTGCCCAGTGCCCCCTACG  
CCGGGCCAGGCCCCCTGACAGCAGTGGATCTGGAGAAAGCTATTGCACAGAACGCAGTG  
GGTACCTGGCAGATGGGCCAGCCAGTGACTCCAGCTCAGGGCCCTGTCATGGCTCTTCC  
AGTGACTCTGTGGTCAACTGCACGGACATCAGCCTACAGGGGGTCCATGGCAGCAGTTC  
30 TACTTTCTGCAGCTCCCTAAGCAGTGACTTTGACCCCCTAGTGTACTGCAGCCCTAAAG  
GGGATCCCCAGCGAGTGGACATGCAGCCTAGTGTGACCTCTCGGCCTCGTTCCCTGGAC  
TCGGTGGTGCCACAGGGGAAACCCAGGTTTCCAGCCATGTCCACTACCACCGCCACCG

GCACCACCACTACAAAAAGCGGTTCCAGTGGCATGGCAGGAAGCCTGGCCCAGAAACCG  
GAGTCCCCCAGTCCAGGCCTCCTATTCTCGGACACAGCCCCAGCCAGAGCCACCTTCT  
CCTGATCAGCAAGTCACCGGATCCAACCTCAGCAGCCCCCTTCGGGGCGGCTCTCTAACCC  
ACAGTGCCCCAGGGCCCTCCCTGAGCCAGCCCCTGGCCCAGTTGACGCCTCCAGCATCT  
5 GCCCCAGTACCAGCAGTCTGTTCAACTTGCAAAAATCCAGCCTCTCTGCCCCGACACCCA  
CAGAGGAAAAGGCGGGGGGGTCCCTCCGAGCCCACCCCTGGCTCTCGGCCCCAGGATGC  
AACTGTGCACCCAGCTTGCCAGATTTTTCCCCATTACACCCCCAGTGTGGCATATCCTT  
GGTCCCCAGAGGCACACCCCTTGATCTGTGGACCTCCAGGCCTGGACAAGAGGCTGCTA  
CCAGAAACCCCAGGCCCTGTTACTCAAATTCACAGCCAGTGTGGTTGTGCCTGACTCC  
10 TCGCCAGCCCCCTGGAACCACATCCACCTGGGGAGGGGCCTTCTGAATGGAGTTCTGACA  
CCGCAGAGGGCAGGCCATGCCCTTATCCGCACTGCCAGGTGCTGTCTCGGCCAGCCTGGC  
TCAGAGGAGGAACCTCGAGGAGCTGTGTGAACAGGCTGTGTGAGATGTTTACGGCCTAGCT  
CCAACCAAGAGTGTGCTCCAGATGTGTTTGGGCCCTACCTGGCACAGAGTCCTGCTCCT  
GGGAAAGGAAAGGACCACAGCAAACACCATTCTTTTTTGCCGTACTTCCTAGAAGCACTG  
15 GAAGAGGACTGGTGATGGTGGAGGGTGAGAGGGTGCCGTTTCCTGCTCCAGCTCCAGAC  
CTTGCTCTGCAGAAAACATCTGCAGTGCAGCAAATCCATGTCCAGCCAGGCAACCAGCTG  
CTGCCTGTGGCGTGTGTGGGCTGGATCCCTTGAAGGCTGAGTTTTTTGAGGGCAGAAAGC  
TAGCTATGGGTAGCCAGGTGTTACAAAGGTGCTGCTCCTTCTCCAACCCCTACTTGGTT  
TCCCTCACCCCAAGCCTCATGTTTCATACCAGCCAGTGGGTTTCAGCAGAACGCATGACAC  
20 CTTATCACCTCCCTCCTTGGGTGAGCTCTGAACACCAGCTTTGGCCCCCTCCACAGTAAG  
GCTGCTACATCAGGGGCAACCCTGGCTCTATCATTTTTCTTTTTTGCCAAAAGGACCAG  
TAGCATAGGTGAGCCCTGAGCACTAAAAGGAGGGGTCCCTGAAGCTTTCCCACTATAGT  
GTGGAGTTCTGTCCCTGAGGTGGGTACAGCAGCCTTGGTTCCTCTGGGGGTTGAGAATA  
AGAATAGTGGGGAGGGAAAAACTCCTCCTTGAAGATTTCTGTCTCAGAGTCCCAGAGA  
25 GGTAAGAAAGGAGGAATTTCTGCTGGACTTTATCTGGGCAGAGGAAGGATGGAATGAAGG  
TAGAAAAGGCAGAAATTACAGCTGAGCGGGGACAACAAAGAGTTCTTCTCTGGGAAAAGT  
TTTGTCTTAGAGCAAGGATGGAAAATGGGGACAACAAAGGAAAAGCAAAGTGTGACCCT  
TGGGTTTGGACAGCCCAGAGGCCAGCTCCCCAGTATAAGCCATACAGGCCAGGGACCC  
ACAGGAGAGTGGATTAGAGCACAAGTCTGGCCTCACTGAGTGGACAAGAGCTGATGGGC  
30 CTCATCAGGGTGACATTCACCCAGGGCAGCCTGACCACTCTTGGCCCCCTCAGGCATTA  
TCCCATTTGGAATGTGAATGTGGTGGCAAAGTGGGCAGAGGACCCACCTGGGAACCT  
TTTTCCCTCAGTTAGTGGGGAGACTAGCACCTAGGTACCCACATGGGTATTTATATCT



GAACCAGACAGACGCTTGAATCAGGCACTATGTTAAGAAATATATTTATTTGCTAATA  
TATTTAT (SEQ ID NO:24)

The hypothetical protein encoded by this sequence is listed under GENBANK Accession No.

5 BAA91085, provided below:

**BAA91085 Amino Acid Sequence**

MSGGHQLQLAALWPWLLMATLQAGFGRTGLVLAAAVESERSAEQKAVIRVIPLKMDPTG  
KLNLTLLEGVFAGVAEITPAEGKLMQSHPLYLCNASDDDNLEPGFISIVKLESPRRAPRP  
10 CLSLASKARMAGERGASAVLFDITEDRAAAEQQLQOPLGLTWPVVLINGNDAEKLMEFVY  
KNQKAHVRIELKEPPAWPDYDVWILMTVVGTIFVIIASVLRIRCRPRHSRPDPLQORT  
AWAISQLATRRYQASCRQARGEWPDSGSSCSSAPVCAICLEEFSEGQELRVISCLHEFH  
RNCVDPWLHQHRTCPLCVFNI TEGDSFSQSLGPSRSYQEPGRRLHLIRQHPGHAHYHLP  
AAYLLGPSRSAVARPPRPGPFLPSQEPGMGPRHHRFPRAAHPRAPGEQQRLAGAHQHPYA  
15 QGWGMSHLQSTSQHAAACPVPLRRARPPDSSSGSGESYCTERSGYLADGPASDSSSGPCH  
GSSSDSVVNCTDISLQGVHGSSTFCSSLSSDFDPLVYCSPKGDQPQRVDMQPSVTSRPR  
SLDSVVPTGETQVSSHVHYHRHRHHYKRFQWHGRKPGPETGVPQSRPPIPRTPQPE  
PPSPDQQVTGSNSAAPSGRLSNPQCPRALPEPAPGPVDASSICPSTSSLFNLQKSSLSA  
RHPQRKRRGGPSEPTPGSRPQDATVHPACQIFPHYTPSVAYPWSPEAHPLICGPPGLDK  
20 RLLPETPGPCYSNSQPVWLCLTPRQPLEPHPPGEGPSEWSSDTAEGRPCPYPHCQVLSA  
QPGSEEELEELCEQAV (SEQ ID NO:25)

Candidate 4: GENBANK Accession No. AA813827

**AA813827/IMAGE Clone 1271704 3' mRNA Sequence**

25 TTTTTTTTTTAAACATTAAGATTTTATTACAAACCAGGCATTATATATTTCTTTACTT  
AAGGAATAGATATGAAACAATCTTGGAGTAAAAATTAGAAGGCAACTTGCTTCAAGTTT  
GTACCAAGTCAATCAAGCAGAAACCTGAAGAACCTTGTTTTAAGATGAGAGTCATTTAT  
ACTTGGCAGGCATTTTCTTCCAATGAAAAAATAAAGTCAATGTGCCATTATCTTGACAC  
TTATAAAAATGTTTATAAAAAGCATTTAGGCCATTGATTCTCACAGTTGGCTGAATATT  
30 GGAATCACCTAGATTAAAAAAAATACTAATCCCTATACAACATCCCCAAAATTCAGATT  
TAATTAGTGTAAGTTAGGCCCTGGGCATATAGGCTGTTTTAAATTCCTCGGGTGAGTC  
TAATGTGTA (SEQ ID NO:26)

## IMAGE Clone 1341074 T7 Sequence

CCCNCCNNCCNNNNNNNGNNNNNCTTANCTCGCAGNCANAATTCGGCCACGCAGGGTCGC  
CTTCGCCGCCATGGNACGCCACCGGGCGCTGACAGACCTATGGAGAGTCAGGGTGTGCC  
TCCCGGGCCTTATCGGGCCACCAAGCTGTGGAATGAAGTTACCACATCTTTTCGAGCAG  
5 GAATGCCTCTAAGAAAACACAGACAACACTTTAAAAAATATGGCAATTGTTTCACAGCA  
GGAGAAGCAGTGGATTGGCTTTATGACCTATTAAGAAATAATAGCAATTTTGGTCCTGA  
AGTTACAAGGCAACAGACTATCCAACCTGTTGAGGAAATTTCTTAAGAATCATGTAATTG  
AAGATATCAAAGGGAGGTGGGGATCAGAAAATGTTGATGATAACAACCAGCTCTTCAGA  
TTTCCTGCAACTTCGCCACTTAAACTCTACCACGAAGGTATCCAGAATTGAGAAAAAA  
10 CAACATAGAGAACTTTTCCAAAGATAAAGATAGCATTTTAAATTACGAACTTATCTC  
GTAGAACTCCTAAAAGGCATGGATTACATTTATCTCAGGAAAATGGCGAGAAAATAAAG  
CATGAAATAATCAATGAAAGATCAAGAAAATGCAATTGATAATAGAGAACTAAGCCAGG  
AAGATGTTGAAAGAAGNTTGGGAGATATGTTATTCTGATCCTACCTGCAAACCATTTTA  
AGGTGTGCCCATCCCCTAGAAGNAAGTTCTTAAATCCCAAACCAGGTAATCCCCCAAN  
15 TANTTAATGNACAAACATGGNCCAATACAAGTTAANCCNGGGAGTAGTTNTTACTACAA  
AACCAATTCNGATGACCTTCCCCCACNGGNTNTTTNNCTNGCCATGGAAANGNCCCTAC  
CAAANTGGCCCAANAANNCANTGATTTGGAATAATCCNNCCTTTGGTTGGGATTNNANC  
AAATTGANTCCNAANNATCCCCAAATANTTTNCNAAANNCTCCCTGANCCCNACCTANC  
TTTGGAANTTNCCCAATTNTTTGGCAAACNTTTTGGGGANGGAAAGAATTCTCCGGATT  
20 TNAGCCCTTNTGGCAAAGGNTNCACCTNNNTTNAATTTNAAGANNACACCCTNGGNAA  
ATNTAANGGGGCCCCCNATNTTTNAAATNCGCGGAANAAGNTCCAGGNTCCCNTNT  
TTCCCCCAAATNNNATTGGGATTCCTNACCCCCCAN (SEQ ID NO:27)

## IMAGE Clone 1341074 T3 Sequence

CNNNNNANTGCGGCCGCTCATTTTTTTTTTTTTTTTTTCTCTATGNAAGCAGACTGNAG  
25 NAAGAAGGCACTCAGNTTGATTGGAAGGAATTCAAATTGTTTAAGTGAAGGAATTTTGA  
AGACTGTGGATCATCTTGAATTTTATGTATCCCACTGGATCTATCTGAACTGTGATGT  
AGCCACAAACAACTACCAGGAAATGAAACAAAAATTAAGATGCAACTGTATGACAGTGG  
ACAAAAATAAAACAAAAACAATAGTAAAGTTAAAAAATAAAGCATTACTATAGTATATA  
30 TTGTTAGTATAGTATACACAGTAGTTGCTTAATTCAGAAGCCACTTAAATAGGACACAT  
GCAACATTCGGTTACAAACGTGCAAGACAGATGAGTGGTTTTCCCATTTGTAATATAAC  
TTTAAAAAATTATTTCAACAGCCTAATTAAATGGATTGAGCCAGAATACATTTAAAAAA

TCTGTTCTCAGTCTGCAAGTACTAGAAACCTCATAAATATAAGATAATTGTGGTATAAT  
AAAATACATATATTTGATCTTTGTCCTTGGTACCTGGTATGGAGCTCCTAAAATCCTTG  
AAATTTCTGAATGATAGAAGTCTTTAGTTACTCATAACAAGCCTATTTTCAGCGNTATC  
CTGAGTTTCATGCCTAANGGTAAGTANGGCCNNGGCCATGGGTTTGAATTTTCATCCAC  
5 CAACTACAACCCTTGTGGGGAGGAGAAAGGGNCTAGAAATTNAAGTTCNNTTGGNCCAC  
CAGTGACCCAATGAATTGGGTCCNGTCATGCCTTGGNTANTTAAACCTTCCAATTAAAA  
CNCNTAAAACATGCNAGGCTGANGGGAGTTTTNTAGGGTNNNGGAANCCTTGNATGGGG  
CTGGGNATCCCCGGATTGACCCAGAAANGGTAAAAAAACNCTTNGGCCCCCCCCCCCC  
CCCTNACCCGGGGNCTTGGGAAACCCCTCCCTTTGGCCNNTTNCCTGGAGGNCNACCCTT  
10 TTNAATAAACTAAAAGCCATAGNTAAAGGGGCNTTTTNCTNNTTNCCTGGGAANCTTGN  
ANGGAATTTTTNGACCCNGGNAAGGGGNTTTGAGGGAAANCCCAANTNGGTAAATTGGCN  
GGGCGGGAATTTNNATACCCCCNGAACCCNATTNCNCGGAATTAAAAAAATTTNGGNNC  
GGNCCCCTTTNTNTNNCCAGGGGTNAAANTTCTCNAANNANAAA (SEQ ID NO:28)

15 IMAGE Clone 1676529 T7 Sequence

AGCTCGNAGCCAGATTCGGCAGGAGGAGATTATATGTTTTATTTATCATTGTCTCTGC  
ATATCTGGAACAACGAAAGGCACATAGCAGTTGCTAAATAAATATCTTTTGAATGAATA  
TATGATTGCCTTATACTTCTTTTATATCCCATCTTCTAATAGATTATGAAAACCTAGAA  
TTCAAAATATATATACTGAACAAATGAATGACTGAAGCAATTGGGGATAATATTTAAGG  
20 CAAAACCAAATCTGATAAAATATACACATATTTTAAAAACACATACATATATATAAATA  
GATCAAAAGTGGAAGAAAGAATATATAAAGAGTGCAACATTTGGCAGCTGAGAATTATT  
TCATTGAGTTTTCAAATATTCTTCACATTCTTATACTTAGAAACAAAGAAGTAACCCCA  
AACAACTAATTCATTAGCTAATATCTCAGAACTTGCACATTTGCAGATAAATTTTCTTT  
TAAGAACAGAATTATAGTTTAATCCCTAACACAGCTCAGTTTTCAAATTCAGTAAAT  
25 AAAATTTTAGCACACATCATGATAGCCTTACTGGNATAGCTGTGTTAAAAACAAAAAGT  
ATTTGGTATCATCTATTGTTATGTGCTCTCAATTGAGATCTAGTTAGTTTCCTAAGAGT  
CTCACATTGATANCTATTTTGGGCACTTCCTTACATAATGNGNTTATTTAGAAATACCT  
TATTAATGACAGACTTCCTTTTGAGTAGCTACATTCTCAGATATGGCTNCATTTATCAA  
AGTTCCCNAGGATTACCTAATTTTAATTCCAGTTAGNTATCTAAACTACGGAACCTTN  
30 GGNTTTCCTTAAANTCAACATTGGTTGCCTTGATTGGAAGGNTTGGCNCCCAAAAANGG  
CGGNCNTCCCNCCCCGGGGGTGGNAANTCTTTTCNTGAANNNTNCCAAGGNNAATTTCC  
TCCNGAAANCNGGNTTTAANTTTTTTNCNNTTCCCCCTTNAANGGGAACCCCCGGGT

TTTNAAAAAATTTTTCCCAAAANATTCNNCCNATGGGCCCCTTTGGAAAGGNAAAAAN  
 TTTTTTGTCCCTTAAAAANCCCTGGNAACCNAATTTGGTTNANCAAATANAGGAAGG  
 (SEQ ID NO:29)

5 IMAGE Clone 167529 T3 Sequence

GCGGCCGCTGGGCCTGNGTGTGCGCTTCGCCGCCATGGNCGCCACCGGGCGCTGACAGA  
 CCTATGGAGAGTCAGGGTGTGCCTCCCGGGCCTTATCGGGCCACCAAGCTGTGGAATGA  
 AGTTACCACATCTTTTCGAGCAGGAATGCCTCTAAGAAAACACAGACAACACTTTAAAA  
 AATATGGCAATTGTTTCACAGCAGGAGAAGCAGTGGATTGGCTTTATGACCTATTAAGA  
 10 AATAATAGCAATTTTGGTCCTGAAGTTACAAGGCAACAGACTATCCAAGTGTGAGGAA  
 ATTTCTTAAGAATCATGTAATTGAAGATATCAAAGGGAGGTGGGGATCAGAAAATGTTG  
 ATGATAACAACCAGCTCTTCAGATTTCTTGCAACTTCGCCACTTAAACTCTACCACGA  
 AGGTATCCAGAATTGAGAAAAACAACATAGAGAACTTTTCCAAAGATAAAGATAGCAT  
 TTTTAAATTACGAACTTATCTCGTAGAACTCCTAAAAGGCATGGATTACATTTATCTC  
 15 AGGAAAATGGCGAGAAAATAAAGCATGAAATAATCAATGAAGATCAAGAAAATGCAATT  
 GATAATAGAGAACTAAGCCAGGAAGATGTTGAAGAAGTTTGGGAGATATGTTATTCTGA  
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 GTAATTCCCAATATATAATGTACNACATGGCCAATACANGTAACGTGGGAGTAGTTAT  
 ACTACAAACAAATCAGATGACCTCCCTCACTGGGTATTATCTGCCATGAAGNGCCTAGC  
 20 AAATNGGCCAGAAGCATGATATGNAATAATCCACCTTTGNNGGATTTGACCGANATGTN  
 TTNGAACATCCCGATTATTTCTAAACCCCTGACCNCTNNTACTTTGAAATNANAATTAT  
 TGNAANCTTTGGGNTGCTNCNCCCTTTAAAGGGGTGCCNCCAAGCCTNNGTTNGTGNTG  
 TTACTNCCCCCAANCGAAAAGNNCNCCTTTATGGGTGNTNCCCAAGAACAATNTNN  
 (SEQ ID NO:30)

25 These sequences correspond to hypothetical gene FLJ20354/GENBANK Accession No.  
 No. AK000361.

AK000361 Nucleotide Sequence

30 GTGCCGAGACTCACCCTGCCGCGGCCGCTGGGCCTGAGTGTGCGCTTCGCCGCCATGG  
 ACGCCACCGGGCGCTGACAGACCTATGGAGAGTCAGGGTGTGCCTCCCGGGCCTTATCG  
 GGCCACCAAGCTGTGGAATGAAGTTACCACATCTTTTCGAGCAGGAATGCCTCTAAGAA

AACACAGACAACACTTTAAAAAATATGGCAATTGTTTCACAGCAGGAGAAGCAGTGGAT  
TGGCTTTATGACCTATTAAGAAATAATAGCAATTTTGGTCCTGAAGTTACAAGGCAACA  
GACTATCCAACCTGTTGAGGAAATTTCTTAAGAATCATGTAATTGAAGATATCAAAGGGA  
GGTGGGGATCAGAAAATGTTGATGATAACAACCAGCTCTTCAGATTTCTGCAACTTCG  
5 CCACTTAAAACTCTACCACGAAGGTATCCAGAATTGAGAAAAACAACATAGAGAACTT  
TTCAAAGATAAAGATAGCATTTTTAAATTACGAACTTATCTCGTAGAACTCCTAAAA  
GGCATGGATTACATTTATCTCAGGAAAATGGCGAGAAAATAAAGCATGAAATAATCAAT  
GAAGATCAAGAAAATGCAATTGATAATAGAGAACTAAGCCAGGAAGATGTTGAAGAAGT  
TTGGAGATATGTTATTCTGATCTACCTGCAAACCATTTTAGGTGTGCCATCCCTAGAAG  
10 AAGTCATAAATCCAAAACAAGTAATTCCCCAATATATAATGTACAACATGGCCAATACA  
AGTAAACGTGGAGTAGTTATACTACAAAACAAATCAGATGACCTCCCTCACTGGGTATT  
ATCTGCCATGAAGTGCCTAGCAAATTTGGCCAAGAAGCAATGATATGAATGATCCAACCT  
ATGTTGGATTTGAACGAGATGTATTCAGAACAATCGCAGATTATTTTCTAGATCTCCCT  
GAACCTCTACTTACTTTTGAATATTACGAATTATTTGTAAACATTTTGGTTGTTTGTGG  
15 CTACATCACAGTTTCAGATAGATCCAGTGGGATACATAAAATTCAAGATGATCCACAGT  
CTTCAAATTCCTTCACTTAAACAATTTGAATTCCTTCAAATCAACTGAGTGCCTTCTT  
CTCAGTCTGCTTCATAGAGAAAAAACAAGAAGAATCAGATTCTACTGAGAGACTACA  
GATAAGCAATCCAGGATTTCAAGAAAGATGTGCTAAGAAAATGCAGCTAGTTAATTTAA  
GAAACAGAAGAGTGAGTGCTAATGACATAATGGGAGGAAGTTGTCATAATTTAATAGGG  
20 TTAAGTAATATGCATGATCTATCCTCTAACAGCAAACCAAGGTGCTGTTCTTTGGAAGG  
AATTGTAGATGTGCCAGGGAATTCAGTAAAGAGGCATCCAGTGTCTTTCATCAATCTT  
TTCCGAACATAGAAGGACAAAATAATAAACTGTTTTTAGAGTCTAAGCCCAAACAGGAA  
TTCCTGTTGAATCTTCATTCAGAGGAAAATATTCAAAGCCATTCAGTGCTGGTTTTAA  
GAGAACCTCTACTTTGACTGTTCAAGACCAAGAGGAGTTGTGTAATGGGAAATGCAAGT  
25 CAAAACAGCTTTGTAGGTCTCAGAGTTTGCTTTTAAGAAGTAGTACAAGAAGGAATAGT  
TATATCAATACACCAGTGGCTGAAATTATCATGAAACCAAATGTTGGACAAGGCAGCAC  
AAGTGTGCAAACAGCTATGGAAAGTGAACCTCGGAGAGTCTAGTGCCACAATCAATAAAA  
GACTCTGCAAAGTACAATAGAACTTTAGAAAATTCTTTACTTCCAGCTTCTTCTATG  
TTGACTGGCACACAAAGCTTGCTGCAACCTCATTTAGAGAGGGTTGCCATCGATGCTCT  
30 ACAGTTATGTTGTTTGTACTTCCCCACCAAATCGTAGAAAGCTTCAACTTTTAATGC  
GTATGATTTCCCGAATGAGTCAAATGTTGATATGCCCAAACCTTCATGATGCAATGGGT  
ACGAGGTCAGTATGATACATACCTTTTCTCGATGTGTGTTATGCTGTGCTGAAGAAGT

GGATCTTGATGAGCTTCTTGCTGGAAGATTAGTTTCTTTCTTAATGGATCATCATCAGG  
 AAATTCTTCAAGTACCCTCTTACTTACTAGACTGCTAGTGGATAATAACATCTTGACTA  
 CTTAAAAAAGGGACATATTGAAAATCCTGGAGATGGACTATTTGCTCCTTTGCCTAACT  
 TACTCATACTGTAAGCAGATTAGTGCTCAGGAGTTTGATGAGCAAAAAGTTTCTACCTC  
 5 TCAAGCTGCAATTGCTAGAACTCTTTAGAAAATATTATTAAAATACAGGAGTTTACCTT  
 AAAGGAAAAAAAAAAAAACAAAAAAAAAAAAAAAAAAAA (SEQ ID NO:31)

The hypothetical protein encoded by this sequence is contained under GENBANK Accession No. BAA91111, provided below:

10

#### BAA91111 Amino Acid Sequence

MESQGVPPGYPYRATKLWNEVTTSTFRAGMPLRKHRQHFKKYGNCFPAGEAVDWLYDLLRNNSN  
 FGPEVTRQQTIQLLRKFLKNHVIEDIKGRWGSENVDDNNQLFRFPATSPLKTLPRRYPELRK  
 NNIENFSKDKDSIFKLRNLSRRTPKRHGLHLSQENGEKIKHEIINEDQENAIIDNRELSQEDV  
 15 EEVWRYVILIYLTILGVPSLEEVINPKQVIPQYIMYNMANTSKRGVVILQNKSDDLPHWVL  
 SAMKCLANWPRSNMNDPTYVGFERDVFRTIADYFLDLPEPLLTFEYYELFVNILVVCGYIT  
 VSDRSSGIHKIQDDPQSSKFLHLNNLNSFKSTECLLLSSHREKNKEESDSTERLQISNPGF  
 QERCAKKMQLVNLNRNRVSANDIMGGSCHNLIGLSNMHDLSSNSKPRCCSLEGIVDVPGNSS  
 KEASSVFHQSPNIEGQNNKLFLESKPKQEFLLNLHSEENIQKPFSAFGKRTSTLTVDQEE  
 20 LCNGKCKSKQLCRSQSLLLRSTRNSYINTPVAEIIKPNVGGGSTSVQTAMESELGESSA  
 TINKRLCKSTIELSENSLLPASSMLTGTQSLQLPHLVAIDALQLCCLLLPPPNNRRKLQLL  
 MRMISRMSQNVDMPKLHDAMGTRSLMIHTFSRCVLCCAEVDLDELLAGRLVSFLMDHHQEII  
 LQVPSYLLDC (SEQ ID NO:32)

25 'Electronic Northern' (E-Northern) depicting gene expression profiles of the above  
 described sequences were determined using the Gene Logic (Gaithersburg, Maryland)  
 datasuite. *See* Figures 2-5. The expression of candidate 3 in normal and malignant human  
 tissues was further investigated by PCR experiments using commercially available human  
 cDNA panels and cDNA samples prepared in-house from human tissues and cell lines. *See*  
 30 Figures 6A-6B and 7A-7B.

Expression of Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was measured in these

experiments as a control for cDNA integrity. GAPDH is a housekeeping gene expressed abundantly in all human tissues. The following primers were used to amplify a 482 base pair product of the GAPDH gene:

5 ' ACCACAGTCCATGCCATCAC 3 ' (SEQ ID NO:56)

5 5 ' TCCACCACCTGTTGCTGTA 3 ' (SEQ ID NO:57)

The following primers were used to amplify a 507 base pair product of the candidate 3 gene:

5 ' TCCCACCCGCTGTACCTGTGC 3 ' (SEQ ID NO:58)

5 ' CCTGCAGCTGGCCTGGTACCT 3 ' (SEQ ID NO:59)

10

Colon tumor samples were obtained from Grossmont Hospital in La Mesa, California. Colorectal cancer cell line HCT116 was obtained from the American Type Culture Collection (ATCC, Manassas, Virginia). RNA was prepared from frozen tissue sections using the RNEasy® Maxi kit (Qiagen, #75162) or from fresh HCT116 cells using the RNEasy® Mini kit (Qiagen, #74104). For each sample, 2.5µg RNA was first treated with DNase I (Amplification Grade, Invitrogen #18068-015), then reverse transcribed using the SUPERScript® First Strand Synthesis System for RT-PCR (Invitrogen # 12371-019). For PCR, 1/25 of the reverse transcriptase (RT) reaction was used to screen for candidate 3, and 1/50 was used for GAPDH. The positive control for candidate 3 was IMAGE 2324560, obtained from the ATCC. The following primers were used to amplify a 415 base pair product of the candidate 3 gene:

15

20

5 ' GGAAGATCTGTTGAAGTGCATTGCTGCAGCTGGTAG 3 ' (SEQ ID NO:60)

5 ' CGCCATCCGAGCCTTGCTAGCCAG 3 ' (SEQ ID NO:61)

25

### EXAMPLE 3

Using the same technology employed in Example 1 to identify the CICO genes, the following sequences were identified as differentially expressed in colon cancer:

#### bs421ms433-258

30

At the +2 PCR stage, bs421ms433-258 was found to be overexpressed in malignant colon compared to normal colon (Figure 1). This peak was purified and amplified by PCR using the

linkers with three additional nucleotides (+3 PCR). The +3 peaks were purified and sequenced.

**bs421ms433-258 Nucleotide Sequence**

5 GATCTCACTCAGCAGACAGCAGCAGCCCGGGAGCCTGAGCTCAGGAGGAACTCTTACCTGGA  
AATTGGGAACTGTATGGAGACTCCAAACTGACTTCTTTCAAAAAACAAAAACAAAAATTTT  
TTTAGCTTTGACAAACACACAAAAGTGGTAATAAAGAGAGCCCTCCTTGTCAACCCAAAATG  
TGAGCCCCCTGTGGCAAAACCACCCCCTACCCCATTA (SEQ ID NO:33)

10 These bases correspond to the 3'UTR and some of the final coding exon of the hypothetical protein bK175E3.C22.6, , the sequence of which is set forth below:

**bK175E3.C22.6 Nucleotide Sequence**

cggccgcggggcccggcgcgggcgggccaaggagacggcggttcgtggag  
15 gtggtgctgttcgagtcgagcccaagcgggcgattacaccacctacaccac  
cggcctcacgggccgcttctcgcgggccggggccacgctcagcgccgagg  
gcgagatcgtgcagatgcacccactgggcctatgtaataacaatgacgaa  
gaggacttgtatgaatatggctgggtaggagtggagaagctggaacagcc  
agaattggacccgaaaccatgcctcactgtcctagggaaggccaagcgag  
20 cagtacagcggggagctactgcagtcactctttgatgtgtctgaaaacca  
gaagctattgatcagctgaaccagggtctgaagacccgctcaagaggcc  
ggtggtgtatgtgaagggtgcagatgccattaagctgatgaacatcgtca  
acaagcagaaagtggctcgagcaaggatccagcaccgccctcctcgacaa  
ccactgaatactttgacatggggattttcctggctttcttcgtcgtggt  
25 ctccttgggtctgcctcatcctccttgtcaaaatcaagctgaagcagcgac  
gcagtcagaattccatgaacaggctggctgtgcaggctctagagaagatg  
gaaaccagaaagttcaactccaagagcaagggcgccgggaggggagctg  
tggggccctggacacactcagcagcagctccacgtccgactgtgccatct  
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30 caccggtttcacaggaagtgcgtggaccctggctgctgcagcaccacac  
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cgggtgtgtgtggagaccagcaacctctcacgtgggtcggcagcagaggggtg



accctgccggtgcattaccccgccgcgtgcacaggaccaacgccatccc  
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10 gtgcacgtggccccgcctcccacctggagagcggcagcacgtccagctt  
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 5 agtctctactcgaagttcgtgcaggactaatgcttttaaaatgaggtcta  
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 ttttaaaattttttaatttttcccatagcacttaaaagagattttgtaa  
 gaccttgctgtaaagattttgtaataaaatgggtctaagggctctttttcc  
 10 aacattaccatttttaaaaaatgttttaaaagctagaagacaacttatgt  
 atattctgtatatgtatagcagcacatttcatttatggaaatatgttctc  
 agaataatttatttactaatatatttatcttaagccatgtcttatgttgag  
 agtgtgacattgttggaataatcattgaaaatgactaacacaagaccctg  
 taaatacatgataattgcacacagattttacatatattgcagacaaaaat  
 15 gatttaaaacaagttgtagtcttctatggttttgtaacaaattgtacaca  
 tgactgtataaaaaaaaatacaattttatcaagtatgtgttata (SEQ ID NO:34)

The above sequence encodes the following protein:

20 bK175E3.C22.6 Amino Acid Sequence  
 MHPLGLCNNNDEEDLYEYGWVG VVKLEQPELDPKPCLTVLGKAKRAVQRG  
 ATAVIFDVSENPEAIDQLNQGS EDPLKRPV VYVKGADAIKLMNIVNKQKV  
 ARARIQHRPPRQPT EYFDMGIFLAFFV VVSLVCLILLVKIKLKQRRSQNS  
 MNRLAVQALEKMETRKFNSKSKGRREGSCGALDTLSSSSTSDCAICLEKY  
 25 IDGEELRVIPCTHRFHRKCVDPWLLQHHTCPHCRHNIIEQKGNPSAVCVE  
 TSNLSRGRQQRVTLPVHYPGRVHRTNAIPAYPTRTSMDSHGNPVTLLTMD  
 RHGEQSLYSPQTPAYIRSY PPLHLDHSLAAHRCGLEHRAYS PAHPFRRPK  
 LSGRSFSKAACFSQYETMYQHYYFQGLSYPEQEGQSPPSLAPRGPARAFP  
 PSGSGSLLFP TVVHVAPP SHLESGSTSSFSCYHGHRSVCSGYLADCPGSD  
 30 SSSSSSSGQCHCSSSDSV DCTEVS NQGVY GSCSTFRSSLSDYDPFIYR  
 SRSPCRASEAGGSGSSGRGPALCFEGSPPEELPAVHSHGAGRGEWPWGP  
 ASPSGDQVSTCSLEMNYS SNSSLEHRGPNSSTSEVGLEASPGAAPDLRRT

WKGGHELPSACCCEPQPSAGPSAGAAGSSTLFLGPHLYEGSGPAGGEP  
 QSGSSQGLYGLHPDHLPRTDGVKYEGLPCCFYEEKQVARGGGGSGCYTE  
 DYSVSVQYTLTEEPFPGCYPGARDLSQRIPPIPEDVDCDLGLPSPDCQGTH  
 SLGSWGGTRGPDTPRPHRGLGATREEERALCCQARALLRPGCPPEEAGAV  
 5 RANFPSALQDTQESSTTATEAAGPRSHSADSSSPGA (SEQ ID NO:35)

This protein contains a transmembrane domain as determined by SMART (shown below),  
 SOSUI, and TmPred. SMART also predicts that this protein contains a RING domain, which  
 is a zinc finger domain involved in protein: protein interactions. The structure of the protein  
 10 is depicted schematically below:



#### EXAMPLE 4

Using the Gene Logic database and the methods described generally in Example 2, the  
 15 following additional DNA sequences were identified as being overexpressed in colon tumor  
 tissue:

#### AA781143/Hs19 11415 28 1 1699a

Fragment AA781143 was upregulated 4.16-fold in the colon samples when compared to  
 20 mixed normal tissue. E-Northern analysis of this fragment demonstrates that it is expressed  
 in 69% of the colon tumors with greater than 50% malignant cells and shows little or no  
 expression in normal tissues. See Figure 8.

#### AA781143 Nucleotide Sequence

25 TTGTCTTCTACGACCAGCTGAAGCAAGTGATGAATGCGTACAGAGTCAAGCCGGCCGTCTTT  
 GACCTGCTCCTGGCTGTTGGCATTGCTGCCTACCTCGGCATGGCCTACGTGGCTGTCCAGGT  
 GAGCAGTGCCCAGGCTCAGCACTTCAGCCTCCTCTACAAGACCGTCCAGAGGCTGCTCGTGA  
 AGGCCAAGACACAGTGACACAGCCACCCCCACAGCCGGAGCCCCCGCCGCTCCACAGTCCCT  
 GGGGCCGAGCACGAGTTGGNAGGGGACCCTCTTCTCCCGTCNTGCCNTCGGGTTGCCCCGCT  
 30 CCTCCAGAGACTTNNCAAGGGCCCATCACCCTGGCCTCTGGGCACTTGTGCTGAGACTCTG

GGACCCAGGCAGCTGCCACCTTGTACCATGAGAGAATTTGGGGAGTGCTTGCATGCTAGCC  
AGCAGGCTCCTGTCTGGGTGCCACGGGGCCAGCATTTTGGAGGGAGCTTCCTTCCTTCCTTC  
CTGGACAGGTCGTCATGATGGATGCACTGACTGACCGTCTGGGGCTCAGGCTGGTGTGGGAT  
GCAGCCGGCCG (SEQ ID NO:36)

5

The GeneLogic database calls this protein "hypothetical protein from EUROIMAGE  
2021883."

EUROIMAGE 2021883 Nucleotide Sequence

10 CCAGAGTTTGTCTTCTACGACCAGCTGAAGCAAGTGATGAATGCGTACAGAGTCAAGCCGGC  
CGTCTTTGACCTGCTCCTGGCTGTTGGCATTGCTGCCTACCTCGGCATGGCCTACGTGGCTG  
TCCAGCACTTCAGCCTCCTCTACAAGACCGTCCAGAGGCTGCTCGTGAAGGCCAAGACACAG  
TGACACAGCCACCCCCACAGCCGGAGCCCCCGCCGCTCCACAGTCCCTGGGGCCGAGCACGA  
GTGAGTGGACACTGCCCCGCCGCGGGCGGCCCTGCAGGGACAGGGGCCCTCTCCCTCCCCGG  
15 CGGTGGTTGGAACACTGAATTACAGAGCTTTTTTCTGTTGCTCTCCGAGACTGGGGGGGGAT  
TGTTTCTTCTTTTCTTGTCTTTGAACTTCCTTGGAGGAGAGCTTGGGAGACGTCCCGGGGC  
CAGGCTACGGAATTGCGGACGAGCCCCCAGTCTTGGGAGCCGGCCGCCCTCGGTCTGGTGT  
AAGCACACATGCACGATTAAAGAGGAGACGCCGGGACCCCCTGCCCGATCGCGCGCGGCCTC  
CGCCACCGCCTCCTGCCGCAAGGGGCCTGGACTGCAGGCCTGACCTGCTCCCTGCTCCGTG  
20 TCTGTCCTAGGACGTCCCTCCCGCTCCCGATGGTGGCGTGGACATGGTTATTTATCTCTG  
CTCCTTCTTGCCTGGAGGAGGGCAGTGCCAGCCCTGGGGTTCTGGGATTCCAGCCCTCCTGG  
AGCCTTTTGTTCCTCATGTGGTCTCAGTGACCCGTCCCCCTGACAGTGGGCTCGGGGAGCTG  
CATCACCAGCCTTCCCCTTCTCCGACTGCAGGGTCTGATGTCATCATTGACAGCCTTTGCT  
TCGTGGGGGCCTGGCAGGGCCCCCTGCCTCCCCGACCCCGACCCACTGCAAATCCCCGTTC  
25 CCTGCACTCCTCTTCTCCCAGCCCATCCCTCCGGCCCCCTGTGCCTCTGCGGCCCCAGCCCAG  
CTCCCAGGGCCGTCACCTGCTTGGCCCTGGCCCAGCTCCCTGCCCTGAGTCCTGAGCCAGTG  
CCTGGTGTTCCTGGGCTCGGTACTGGGCCCCAGGCCATCCAGGCTTTGCCACGGCCAGTT  
GGTCCTCCCTGGGGAAGTGGGTGCGGGTGGAGTACTGGGAGGCAGGAGGTGGCCCCGGGGAGG  
CCTTGTGGCTCCTCCCCTCGCTCCTCGCCCTGGGCCTCAGCTTCCTCATCAATAGAAAGGAT  
30 GTGTTTCGGGGTGGGGGCGTCAGGTGAGAACGTTTGCTGGGAAGGAGAGGACTTGGGGCATGG  
CCTCTGGGGCCACCCTTCCTGGAAGTCTCAGAGAGGAAGGTCCGGGCCCTCGGGAAGCCTTGA  
CAGAACCTCCACCCCGCAGACCAGGCGTCGTGTGTGTGTGGGAGAGAAGGAGGCCCGTGT

GAGCTCAGGGAGACCCCGGTGTGTCCGTTCTTAGCAATATAACCTACCCAGTGCGTGCCGAG  
CAGGCTTGGTGGGGAAGGGACTTGAGCTGGGCAAGTCCTGGCCTGGCACCCGAGCCGTCTC  
CCTTCCGTGGCCCAGGGAGGTGTTTGCTGTCCGAAGGACCTGGGCCGGCCCATGGGAGCCTG  
GGGTTCTGTCCAGATAGGACCAGGGGGTCTCACTTTGGCCACCAGTTCTTCGGCCAGCACCT  
5 CTGCCCTCCAGAACCTGCAGCCTGGAGGGGTGAGGGGACAACCACCCCTCTTTCCTCCAGGT  
TGGCAGGGGACCCTCTTCTCCCGTCTGCCCTGCGGGTTGCCCGCCTCCTCCAGAGACTTGCC  
CAAGGGCCCATCACCACTGGCCTCTGGGCACTTGTGCTGAGACTCTGGGACCCAGGCAGCTG  
CCACCTTGTCACCATGAGAGAATTGGGGAGTGCTTGCATGCTAGCCAGCAGGCTCCTGTCT  
GGGTGCCACGGGGCCAGCATTTTGGAGGGAGCTTCCTTCCTTCCTTCCTTGACAGGTTCGTCA  
10 TGATGGATGCACTGACTGACCGTCTGGGGCTCAGGCTGGTGTGGGATGCAGCCGGCCGATGA  
GAAAATAAAGCCATATTGAATGAT (SEQ ID NO:37)

### EUROIMAGE 2021883 Amino Acid Sequence

15 (SEQ ID NO:38)

The protein set forth above contains one TM (transmembrane domain) by SMART, SOSUI, and TmPred prediction programs. However, the BLAST database and EST sequences suggest that the following alternative nucleotide and protein sequences correspond to

20 AA781143:

**Hs19 11415 28 1 1699.a Nucleotide Sequence**

gcaaggtcacgtcctgtccccacctttcgccccctcaccctagctcccca  
acgccaaagacaaggttaagaaagtgatatcgcgaaatagtttttaag  
25 cattttattgcattttatgacttggagtttatgtgaaacctcaacggtat  
tagccgaacagcctgccgcaccttccgggagttccagagtgggcctacaa  
ctcccacagggctccgcgagcgccggacggacggactacaattcccgaca  
ggcagcgcggtggcgggcggttcgccgcggtgccacaggacctcagg  
gcgagtgcgggctgccccgcgcggcgccccgcaggaccccgcggtaccc  
30 atgccgaggtgagtcgcgggagccgcgcgcgcgcgcgtcccggtcccagc  
tgccgccccgcgcggccccgcgcggccaggATGCTGGAGGAAGCGGGC  
GAGGTGCTGGAGAACATGCTGAAGGCGTCTTGTCTGCCGCTCGGCTTCAT

CGTCTTCCTGCCCCGCTGTGCTGCTGCTGGTGGCGCCGCGCTGCCTGCCG  
CCGACGCCGCGCACGAGTTCACCGTGTACCGCATGCAGCAGTACGACCTG  
CAGGGCCAGCCCTACGGCACACGGAATGCAGTGCTGAACACGGAGGCGCG  
CACGATGGCGGCGGAGGTGCTGAGCCGCGCTGCGTGCTCATGCGGCTAC  
5 TGGACTTCTCCTACGAGCAGTACCAGAAGGCCCTGCGGCAGTCGGCGGGC  
GCCGTGGTCATCATCCTGCCCAGGGCCATGGCCGCGCTGCCCCAGGACGT  
CGTCCGGCAATTTCATGGAGATCGAGCCGGAGATGCTGGCCATGGAGACCG  
CCGTCCCCGTGTACTTTGCCGTGGAGGACGAGGCCCTGCTGTCTATCTAC  
AAGCAGACCCAGGCTGCCTCCGCCTCCAGGGCTCCGCCTCTGCTGCTGA  
10 AGTACTGCTGCGCACGGCCACTGCCAACGGCTTCCAGATGGTCACCAGCG  
GGGTACAGAGCAAGGCCGTGAGTGACTGGCTGATTGCCAGCGTGGAGGGG  
CGGCTGACGGGGCTGGGCGGAGAGGACCTTCCCACCATCGTCATCGTGGC  
CCACTACGACGCCTTTGGAGTGGCCCCCTGGCTGTCGCTGGGCGCGGACT  
CCAACGGGAGCGGCGTCTCTGTGCTGCTGGAGCTGGCACGCCTCTTCTCC  
15 CGGCTCTACACCTACAAGCGCACGCACGCCGCCTACAACCTCCTGTTCTT  
TGCGTCTGGAGGAGGCAAGTTTAACTACCAGGGAACCAAGCGCTGGCTGG  
AAGACAACCTGGACCACACAGACTCCAGCCTGCTTCAGGACAATGTGGCC  
TTCGTGCTGTGCCTGGACACCGTGGGCCGGGGCAGCAGCCTGCACCTGCA  
CGTGTCCAAGCCGCCTCGGGAGGGCACCCTGCAGCACGCCTTCCTGCGGG  
20 AGCTGGAGACGGTGGCCGCGCACCAAGTTCCTGAGGTACGGTTCTCCATG  
GTGCACAAGCGGATCAACCTGGCGGAGGACGTGCTGGCCTGGGAGCACGA  
GCGCTTCGCCATCCGCCGACTGCCCGCCTTCACGCTGTCCCACCTGGAGA  
GCCACCGTGACGGCCAGCGCAGCAGCATCATGGACGTGCGGTCCCGGGTG  
GATTCTAAGACCCTGACCCGTAAACACGAGGATCATTGCAGAGGCCCTGAC  
25 TCGAGTCATCTACAACCTGACAGAGAAGGGGACACCCCCAGACATGCCGG  
TGTTACAGAGCAGATGCAGATCCAGCAGGAGCAGCTGGACTCGGTGATG  
GACTGGCTCACCAACCAGCCGCGGGCCGCGCAGCTGGTGGACAAGGACAG  
CACCTTCCTCAGCACGCTGGAGCACCACTGAGCCGCTACCTGAAGGACG  
TGAAGCAGCACCACGTCAAGGCTGACAAGCGGGACCCAGAGTTTGTCTTC  
30 TACGACCAGCTGAAGCAAGTGATGAATGCGTACAGAGTCAAGCCGGCCGT  
CTTTGACCTGCTCCTGGCTGTTGGCATTGCTGCCTACCTCGGCATGGCCT  
ACGTGGCTGTCCAGCACTTCAGCCTCCTCTACAAGACCGTCCAGAGGCTG



CTCGTGAAGGCCAAGACACAGTGAcacagccacccccacagccggagccc  
ccgccgctccacagtccctggggccgagcacgagtgagtggacactgccc  
cgccgcgggcgggccctgcagggacaggggccctctccctccccggcggtg  
gttggaaactgaattacagagcttttttctgttgctctccgagactggg  
5 gggggattgtttcttcttttcccttgtctttgaacttccttggaggagagc  
ttgggagacgtcccggggccaggctacggacttgcggaacgagccccccag  
tcctgggagccggccgccctcggtctggtgtaagcacacatgcacgatta  
aagaggagacgcccgggacccccctgccgatcgcgcgcgccctccgcccac  
cgccctctgccgcaagggggcctggactgcaggcctgacctgctccctgct  
10 ccgtgtctgtcctaggacgtcccctcccgtccccgatggtggcgtggac  
atggttatattatctctgctccttcttgccctggaggaggcagtgccagcc  
ctgggggttctgggattccagccctcctggagccttttggtcccatgtgg  
tctcagtgacctgtccccctgacagtgggctcggggagctgcatcaccca  
gccttcccccttctccgactgcagggtctgatgtcatcattgacagccttt  
15 gcttcgtgggggcctggcaggggccctgcctccccgacccccgacccact  
gcaaattcccgttcccctgactcctcttctcccagcccatccctccggc  
ccctgtgcctctgcggccccagcccagctcccaggggccgtcacctgcttg  
gccctggcccagctccctgccctgagtcctgagccagtgccctggtgtttc  
ctgggctcggtactggggccccaggccatccaggctttgccacggccagt  
20 tggctcctccctggggaaactgggtgcgggtggagtactgggaggcaggagg  
tgggccggggaggccttgtggctcctcccctcgctcctcgccctgggcct  
cagcttcctcatcaatagaaaggatgtgttcgggggtgggggcgtcaggtg  
agaacgtttgctgggaaggagaggacttggggcatggcctctggggccac  
ccttcctggaactcagagaggaagggtccgggccctcggaagccttgga  
25 agaaccctccaccccgacagaccaggcgctcgtgtgtgtgtgggagagaagg  
aggcccgtgttgagctcaggagacccccggtgtgtccgttcttttagcaat  
ataacctaccagtgctgcgagcaggcttgggtggggaagggacttgag  
ctgggcaagtcctggcctggcaccgcagccgtctcccttccgtggccca  
gggaggtgtttgctgtccgaaggacctggggccggcccatgggagcctggg  
30 gttctgtccagataggaccagggggtctcactttggccaccagttcttcg  
gccagcacctctgccctccagaacctgcagcctggaggggtgaggggaca  
accacccctctttcctccagggttggcaggggaccctcttctcccgtctgc

cctgcggggttgcccgctcctccagagacttgcccaagggcccatcacca  
 ctggcctctgggcacttgtgctgagactctgggacccaggcagctgccac  
 cttgtcaccatgagagaatttggggagtgcttgcctgctagccagcaggc  
 tcctgtctgggtgccacggggccagcattttggaggagcttccttcctt  
 5 ccttcctggacaggtcgtcatgatggatgcactgactgaccgtctggggc  
 tcaggctggtgtgggatgcagccggccgatgagaaaataaagccatattg  
 aatgatcg (SEQ ID NO:39)

#### Hs19\_11415\_28\_1\_1699.a Amino Acid Sequence

10 MLEEAGEVLENMLKASCLPLGFIVFLPAVLLLVPPLPAADAAHEFTVYR  
 MQQYDLQGQPYGTRNAVLNTEARTMAAEVLSRRCVLMRLLDfsyeqyqka  
 LRQSAGAVVILPRMAAVPQDVVRQFMEIEPEMLAMETAVPVYFAVEDE  
 ALLSIYKQTQAASASQGSASAAEVLLRTATANGFQMVTSGVQSKAVSDWL  
 IASVEGRLTGLGGEDLPTIVIVAHYDAFGVAPWLSLGADSNGSGVSVLLE  
 15 LARLFSRLYTYKRTHAAYNLLFFASGGGKFNYQGTRWLEDNLDHTDSSL  
 LQDNVAFVLCLDTVGRGSSLHLHVS KPPREGTLQHAFLRELETVAAHQFP  
 EVRFSMVHKRINLAEDVLAWEHERFAIRRLPAFTLSHLESHRDGQRSSIM  
 DVRSRVDSKTLTRNTRIIAEALTRVIYNLTEKGTPPDMPVFTEQMQUIQE  
 QLDSVMDWLTNQPRAAQLVDKDSTFLSTLEHHLSRYLKDV KQHVKADKR  
 20 DPEFVFYDQLKQVMNAYRVKPAVFDLLLAVGIAAYLGMAVYVAVQHFSLLY  
 KTVQRLLVKAKTQ (SEQ ID NO:40)

GENBANK also identifies RefSeq Loc56926 as corresponding to AA781143, which  
 nucleotide and protein sequences are set forth below:

25 RefSeq Loq56926 Nucleotide Sequence  
 GGCGAGGTGCTGGAGAACATGCTGAAGGCGTCTTGCTGCGGCTCGGCTTCATCGTCTTCCT  
 GCCCGCTGTGCTGCTGCTGGTGGCGCCGCGCTGCCTGCCGCCGACGCCGCGCACGAGTTCA  
 CCGTGTACCGCATGCAGCAGTACGACCTGCAGGGCCAGCCCTACGGCACACGGAATGCAGTG  
 30 CTGAACACGGAGGCGCGCACGATGGCGGCGGAGGTGCTGAGCCGCGCTGCGTGCTCATGCG  
 GCTACTGGACTTCTCCTACGAGCAGTACCAGAAGGCCCTGCGGCAGTCGGCGGGCGCCGTGG  
 TCATCATCCTGCCAGGGCCATGGCCGCGTGCCCCAGGACGTCGTCCGGCAATTCATGGAG

ATCGAGCCGGAGATGCTGGCCATGGAGACCGCCGTCCCCGTGTACTTTGCCGTGGAGGACGA  
GGCCCTGCTGTCTATCTACAAGCAGACCCAGGCTGCCTCCGCCTCCCAGGGCTCCGCCTCTG  
CTGCTGAAGTACTGCTGCGCACGGCCACTGCCAACGGCTTCCAGATGGTCACCAGCGGGGTA  
CAGAGCAAGGCCGTGAGTGACTGGCTGATTGCCAGCGTGGAGGGGCGGCTGACGGGGCTGGG  
5 CGGAGAGGACCTTCCCACCATCGTCATCGTGGCCCACTACGACGCCTTTGGAGTGGCCCCCT  
GGCTGTCGCTGGGCGCGGACTCCAACGGGAGCGGCGTCTCTGTGCTGCTGGAGCTGGCACGC  
CTCTTCTCCCGGCTCTACACCTACAAGCGCACGCACGCCGCCTACAACCTCCTGTTCTTTGC  
GTCTGGAGGAGGCAAGTTTAACTACCAGGGAACCAAGCGCTGGCTGGAAGACAACCTGGACC  
ACACAGACTCCAGCCTGCTTCAGGACAATGTGGCCTTCGTGCTGTGCCTGGACACCGTGGGC  
10 CGGGGCAGCAGCCTGCACCTGCACGTGTCCAAGCCGCCTCGGGAGGGCACCCTGCAGCACGC  
CTTCCTGCGGGAGCTGGAGACGGTGGCCGCGCACCAGTTCCCTGAGGTACGGTTCTCCATGG  
TGACAAGCGGATCAACCTGGCGGAGGACGTGCTGGCCTGGGAGCACGAGCGCTTCGCCATC  
CGCCGACTGCCCCGCCTTCACGCTGTCCACCTGGAGAGCCACCGTGACGGCCAGCGCAGCAG  
CATCATGGACGTGCGGTCCCGGGTGGATTCTAAGACCCTGACCCGTAACACGAGGATCATTG  
15 CAGAGGCCCTGACTCGAGTCATCTACAACCTGACAGAGAAGGGGACACCCCCAGACATGCCG  
GTGTTTACAGAGCAGATGCAGATCCAGCAGGAGCAGCTGGACTCGGTGATGGACTGGCTCAC  
CAACCAGCCGCGGGCCGCGCAGCTGGTGGACAAGGACAGCACCTTCCTCAGCACGCTGGAGC  
ACCACCTGAGCCGCTACCTGAAGGACGTGAAGCAGCACCAAGTCAAGGCTGACAAGCGGGAC  
CCAGAGTTTGTCTTCTACGACCAGCTGAAGCAAGTGATGAATGCGTACAGAGTCAAGCCGGC  
20 CGTCTTTGACCTGCTCCTGGCCGTTGGCATTGCTGCCTACCTCGGCATGGCCTACGTGGCTG  
TCCAGCACTTCAGCCTCCTCTACAGGACCGTCCAGAGGCTGCTCGTGAAGGCCAAGACACAG  
TGACACAGCCACCCCCACAGCCGGAGCCCCCGCCGCTCCACAGTCCCTGGGGCCGAGCACGA  
GTGAGTGGACACTGCCCCGCCGCGGGCGGCCCTGCAGGGACAGGGGCCCTCTCCCTCCCCGG  
CGGTGGTTGGAACACTGAATTACAGAGCTTTTTTCTGTTGCTCTCCGAGACTGGGGGGGGAT  
25 TGTTTCTTCTTTTCTTGTCTTTGAACTTCCTTGGAGGAGAGCTTGGGAGACGTCCCGGGGC  
CAGGCTACGGACTTGCGGACGAGCCCCCAGTCCCTGGGAGCCGGCCGCCCTCGGTCTGGTGT  
AAGCACACATGCACGATTAAAGAGGAGACGCCGGGACCCCCCTGCCCGATCGCGCGCGGCCTC  
CGCCCACCGCCTCCTGCCGCAAGGGGCCTGGACTGCAGGCCTGACCTGCTCCCTGCTCCGTG  
TCTGTCTTAGGACGTCCCCCTCCCGCTCCCCGATGGTGGCGTGGACATGGTTATTTATCTCTG  
30 CTCCTTCTTGCCTGGAGGAGGGCAGTGCCAGCCCTGGGGTTCTGGGATTCCAGCCCTCCTGG  
AGCCTTTTGTTCCTCATGTGGTCTCAGTGACCCGTCCCCCTGACAGTGGGCTCGGGGAGCTG  
CATCACCAGCCTTCCCCTTCTCCGACTGCAGGGTCTGATGTCATCGTTGACAGCCTTTGCT

TCGTGGGGGCTGGCAGGGCCCCTGCCTCCCCGACCCCCGACCCACTGCAAACCCCCGTTCC  
CCTGCACTCCTCTTCTCCCAGCCCATCCCTCCGGCCCCCTGTGCCTCTGCGGCCCCAGCCCAG  
CTCCCAGGGCCGTCACCTGCTTGGCCCTGGCCCAGCTCCCTGCCCTGAGTCCTGAGCCAGTG  
CCTGGTGTTCCTGGGCTCGGTACTGGGCCCCCAGGCCATCCAGGCTTTGCCACGGCCAGTT  
5 GGTCTCCCTGGGGAACCTGGGTGCGGGTGGAGTACTGGGAGGCAGGAGGTGGCCCCGGGGAGG  
CCTTGTGGCTCCTCCCCTCGCTCCTCGCCCTGGGCCTCAGCTTCCTCATCAATAGAAAGGAT  
GTGTTCCGGGGTGGGGGCGTCAGGTGAGAACGTTTGCTGGGAAGGAGAGGACTTGGGGCATGG  
CCTCTGGGGCCACCCTTCCTGGAACCTCAGAGAGGAAGGTCCGGGCCCCCGGGAAGCCTTGGA  
CAGAACCCTCCACCCCCGAGACCAGGCGTCGTGTGTGTGTGGGAGAGAAGGAGGCCCGTGT  
10 GAGCTCAGGGAGACCCCGGTGTGTCCGTTCTTTAGCAATATAACCTACCCAGTGCGTGCCGA  
GCAGGCTTGGTGGGGAAGGGACTTGAGCTGGGCAAGTCCTGGCCTGGCACCCGAGCCGTCT  
CCCTTCCGTGGCCCAGGGAGGTGTTTGCTGTCCGAAGGACCTGGGCCGGCCCATGGGAGCCT  
GGGGTTCTGTCCAGATAGGACCAGGGGGTCTCACTTTGGCCACCAGTTCTTCGGCCAGCACC  
TCTGCCCTCCAGAACCTGCAGCCTGGAGGGGTGAGGGGACAACCACCCCTCTTTCCTCCAGG  
15 TTGGCAGGGGACCCTCTTCTCCCGTCTGCCCTGTGGGTGCCCCGCTCCTCCAGAGACTTGC  
CCAAGGGCCCATCACCCTGGCCTCTGGGCACTTGTGCTGAGACTCTGGGACCCAGGCAGCT  
GCCACCTTGTACCATGAGAGAATTTGGGGAGTGCTTGCGATGCTAGCCAGCAGGCTCCTGTC  
TGGGTGCCACGGGGCCAGCATTTTGGAGGGAGCTTCCTTCCTTCCTTCCTGGACAGGTCGTC  
AGGATGGATGCACTGACTGACCGTCTGGGGCTCAGGCTGGTGTGGGATGCAGCCGGCCGATG  
20 AGAAAATAAAGCCATATTGAATGATAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO:49)

## RefSeq Loq56926 Amino Acid Sequence

MLKASCLPLGFIVFLPAVLLLVPPLPAADAAHEFTVYRMQQYDLQGQPYGTRNAVLNTEAR  
TMAAEVLSRRCVLMRLLDfsyeQYQKALRQSAGAVVILPRMAAVPQDVVRQFMEIEPEML  
25 AMETAVPVYFAVEDEALLSIYKQTQAASASQGSASAAEVLLRTATANGFQMVTSGVQSKAVS  
DWLIASVEGRLTGLGGEDLPTIVIVAHYDAFGVAPWLSLGADSNNGSVSVLLELARLFSRLY  
TYKRTHAAYNLLFFASGGGKFNYQGTKRWLEDNLDHTDSSLLQDNVAFVLCCLDTVGRGSSLH  
LHVSKPPREGTLQHAFLRELETVAAHQFPEVRFSMVHKRINLAEDVLAWEHERFAIRRLPAF  
TLSHLESHRDGQRSSIMDVRSRVDskTLTRNTRIIAEALTRVIYNLTEKGTTPDMPVFTEQM  
30 QIQQEQLDSVMDWLTNQPRAAQLVDKDSTFLSTLEHHLSRYLKDVKQHHVKADKRDPEFVFY  
DQLKQVMNAYRVKPAVFDLLLAVGIAAYLGMAVAVQHFSLLYRTVQRLLVKAKTQ (SEQ  
ID NO:50)

The RefSeq Loq56926 protein has a transmembrane domain as predicted by SOSUI and TmPred. It also has both a signal peptide and a transmembrane domain predicted by SMART, suggesting that this is a type I membrane protein with the majority of the protein being extracellular.

5

The expression of Loc56926 in normal and malignant human tissues was further investigated by PCR experiments using commercially available human cDNA panels and cDNA samples prepared in-house from human tissues and cell lines. See Figures 9A-9B, 10A-10B, 11A-11B, and 12A-12B. Expression of Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was measured in these experiments as a control for cDNA integrity. GAPDH is a housekeeping gene expressed abundantly in all human tissues. The following primers were used to amplify a 482 base pair product of the GAPDH gene:

10

5 ' ACCACAGTCCATGCCATCAC 3 ' (SEQ ID NO:62)

5 ' TCCACCACCCTGTTGCTGTA 3 ' (SEQ ID NO:63)

15

For expression studies, malignant colon samples were obtained from Analytical Pathology Medical Group and frozen within thirty minutes of surgery. The HCT116 colon cancer cell line was obtained from American Type Culture Collection (ATCC of Manassas, Virginia.). RNA was extracted from the samples using RNEASY® Maxi Kit (Qiagen #75162) or from fresh HCT116 cells using the RNEASY® Mini kit (Qiagen, #74104) according to the manufacture's instructions and reverse transcribed into cDNA using SUPERScript® II Kit (Invitrogen # 12371-019). The positive control for Loc56926 IMAGE clone 4428206 was obtained from the ATCC. Primers used to amplify a 283 base pair product of Loc56926 were:

20

5 ' AATGCAGTGCTGAACACGGAG 3 ' (SEQ ID NO:64)

5 ' TCTGCTTGTAGATAGACAGCAGG 3 ' (SEQ ID NO:65)

25

#### AW779536

In a comparison of malignant colon samples containing greater than 50% malignant cells in the sample against mixed normal tissues, fragment AW779536 was upregulated 3.7 fold. Northern analysis shown in Figure 13 demonstrates that the fragment is expressed in 77% of the tumors and poorly expressed in normal tissue.

30

## AW779536 Nucleotide Sequence

TTCTTCCTGTGTTACAATTACCCTGTTTCTGATTACTACAGCCCAACCCGGGCGGACACCAC  
 CACCATTCTGGCTGCCGGGGCTGGAGTGACCATAGGATTCTGGATCAACCATTTCTTCCAGC  
 TTGTATCCAAGCCCGCTGAATCTCTCCCTGTTATTCAAGACATCCCACCGNTCACCACCTAC  
 5 ATGTTAGNNTTGGGTCTGACCAAATTTGCAGTGGGAATTGTGTTGATCCTCTTGGTTTCGTCA  
 GCTTGTACAAAATCTCTCACTGCAAGTATTATACTCATGGTTCNAGGTNGGTCNCCAGGAAC  
 AAGGAGGCCAGGCGGAGACTGGAGATTGAAGTGCCCTACAAGTTTGTTACCTACACATCTGT  
 TGGCATCTGCGCTACAACCTTTGTGCCGATGCTTCACAGGTTTCTGGGATTACCCTGAGTCT  
 CAAACAGTTGGAACTAGCCCACTGGACATGAAAGCCAAGACATAGGAAAGTTATTGGTAGG  
 10 CAAATCTTGACAACTTATTTTTCTTTAACAACAACAAAAGTCATACGGCTGTCTTGCTACT  
 (SEQ ID NO:41)

BLAST searching with this sequence revealed a hypothetical protein predicted by Acembly, Ensembl and Fgenesh++, Hs2\_5283\_28\_1\_1143.b with the following nucleotide sequence:

15 Hs2\_5283\_28\_1\_1143.b Nucleotide Sequence  
 GCTTATGTACAGAAGTACGTCGTGAAGAATTATTTCTACTATTACCTATT  
 CCAATTTTCAGCTGCTTTGGGCCAAGAAGTGTTCTACATCACGTTTCTTC  
 Cattcactcactggaatattgacccttatttatccagaagattgatcatcatatgggttttg  
 20 gtgatgtatattggccaagtggccaaggatgtcttgaagtggccccgtccctcctcccctcc  
 agttgtaaaactggaaaagagactgatcgctgaatatggaatgccatccaccacgccatgg  
 cggccactgccattgccttcaccctccttatctctactatggacagataccagtatccattt  
 gtgttgggactggtgatggcogtggtgttttccaccttggtgtgtctcagcaggctctacac  
 tgggatgcatacggtcctggatgtgctgggtggcgtcctgatcacegcactcctcatcgctcc  
 25 tcacctaccctgctggaccttcacgactgcctggactcggccagccccctcttccccgctg  
 tgtgtcatagttgtgccattcttctctgtgttacaattaccctgtttctgattactacagccc  
 aaccggggcggacaccaccaccattcttggtgcccggggctggagtgaccataggattctgga  
 tcaaccatttcttccagcttgatatccaagcccgtgaatctctccctgttattcagaacatc  
 ccaccactcaccacctacatgttagttttgggtctgaccaaatttgacgtgggaattgtgtt  
 30 gatcctcttggttcgtcagcttgtaaaaaatctctcactgcaagattatactcatggttca  
 aggtggtcaccaggaacaaggaggccaggcggagactggagattgaagtgccttacaagttt  
 gttacctacacatctggttggcatctgcgctacaacctttgtgccgatgcttcacaggtttct

gggattaccctgagtctcaaacagttggaaactagcccactggacatgaaagccaagacata  
ggaaagttattggtaggcaaactcttgacaacttatttttctttaacaacaacaaaaagtc  
acggctgtcttgctactaccagataaatgatgctgctgtgtgaaaggaagaactgtctcata  
gcggtcattgggtcggtccgtgggtgggtgtgtgtacagttgaaccagggttaaagaccata  
5 atccggatctttaaaggcacacaccgcgcccccccccccccccgcccgccctgctcctctc  
gctgttgacgggctttggatctagtcatgggctggcaggaattgtggcctggcttaggaat  
agctatgagccccactgggttctggagagccagtagagatggggtgatctgggaggctggag  
gtagagcctttcttttccgttacaaccttgccatgcatggagttaactgtgcctgggtgggt  
ggtaagatcactctgaaagaaagctcactgtgaagagatgaaaggtggaggcagagctgtga  
10 ggtcatggggaaaagcctgctttccttataagtcctgctgttcattgttggaataaggatctg  
ctcttccttggtttccatgcattttgcaggattccagggtaccattaccacactcttctgaccc  
atgaaaccaactggctgctcacacatcaccaaacagggtgggggttagccttcagcacagg  
ggatacatctgggattcactgagattcctgccctctcctgcttcctagtgggttgggacagg  
ccctctgcccacgtcagcagttttttgctttcatacaaacctggaaggcactggcatctgc  
15 ctaggaaagtggatctgtgaagaacagatgaactcaatcctttctggagtctgacaaagaag  
ggataggcttccttgacattgcctgtcctgacaaggcctccctgacattactcctccaattt  
cacagttaccttctgtaaattctattttctcatctactgaatagaatcaggcgccctttttgt  
cttcccacctcttatctcttggaattttaaggggaattaatgcaagaacaactttagtgtc  
tcttgggaaaacaagccaaccaaatacaaaaaccatttaagcctactagggtgagtcctctta  
20 acatgggaaggcgatgattatgcaaacaccggagttccctcctcttcagttcctaagaataa  
agaacagggtatcaagaactttctttaagttagtgtactatagtttaacaaagtatccattg  
aagtttagtgctgtaggactgagccagtgctttatcaacccaacacatcatcaccatgtgc  
atactctagaaaaaaaaatagcttccttaaaagttacagaggctcttaacgtgttaaaaccg  
aaaaatcacattttttcttgatttcaaatatgttctacggccttactgttgggatgatattta  
25 gtatgtaacttagcattccaattttctcaagaatttttaggccgggtgcggtggctcatgcct  
gtaatcccagcactttgggaggccgaggtgggaggaccacgaggtcaggagatcgagaccat  
cctggctaacacgggtacccgtctctactgaaaatacaaaaaaattagccggacgtgggtgga  
gggcgctgtagtcccagctactcaggaggctgaggcaggagaatggcgtgaaccgggtgag  
cggagcttgagtgagccgagattgcgccactgcactccagcctgggagcagagcgagact  
30 ctctcaaaaaaaaaaaaaaaaaagaatttttagcaaaacatcctgtttttacttaaaattcttct  
catatttattatagttagaaggcaaagatcaagatgacctgccgtttgactgcttttacatc  
aaactctgccagtatgtgcagcacaactcaggggaagggccttagcttacagggtactccca

gccttcacatctgcccctgcagagcagtggtgtcagccggatgcggcacttttctgtattttc  
 atccacacagctgccagccagagttcgcaacactggatatttacaccaaataattgtgggtt  
 gacttgtctgaagccagctgacaaaaggatcagcttttcccacttgtattttttaaaaagag  
 ggattgtgatcattgtcacagagtggtgtgctggcctctcatatatatgatatatatatatca  
 5 ttttatatatatatatatatcatatacataatttttactgctgtctctagttttaagtccca  
 acaataggaaggccgatcagctatattgatataatttaaggctgtacttaactaatttgggct  
 gaggatgaatatatcagccacagcacattaaagaatgagccaaggatttgtcatgggtgggtc  
 actttttaaagtatttgattactgcaactggagaatgaaaagtgtatattggtgacgccaac  
 ctcagtttctgagcactcctgctctgtgggtgagaatcagacaaaaattcatcggggtgaaaa  
 10 aggcattacctgattcacacccttgtcttgctagccctcttccattcattttctcacacagca  
 ctttgcctctgttaaactctctctgtctcagaccattgcttgccccttcaaagggtatggt  
 tcaggctcctttcaagacatttggagtttctctctggtgggaagagagccccctactgggttg  
 gcttcagtctagggtccaccatccctctcgatctggcatcttgagattaatttaaaaggcaa  
 gctcaccacaatgtaagcctatggtctggccaaccttgcttttgggaactgtgacaccaaag  
 15 cccccaggactatctgcctctccaggagccagatagaatgacatgccttttccctaattgtc  
 cacattccaccccccaaccactgccactgtgggccaagccatccatcttgcaatcttcatct  
 aaaacagctctcattttcatgccagttttgctcaaacctgcaccgtcacaagatattcagaag  
 atgaaaacgtagaagacaccctgaattaaaaacacttacatagcagtggtggaattactc  
 caaaacgtgccagtgatcgactgtaacatgggattttctcacccaaataggcaactcatg  
 20 cttcctgagtgtaatcaaagcatgtggtgttttggggccatatgcaccagggtttctatttta  
 gaaaccttcagctgtcttgcttatgtactgtatgtaaatttattcttttttaaaaatcacttt  
 tatttgattttgacttattaaatgcttttaaagccag (SEQ ID NO:42)

The amino acid sequence of Hs2\_5283\_28\_1\_1143.b is set forth below:

25

#### Hs2\_5283\_28\_1\_1143.b Amino Acid Sequence

AYVQKYVVKNYFYYYLFQFSAALGQEVFYITFLPFTHWNIDPYLSRRLII  
 IWVLVMIYIGQVAKDVLKWPRPSSPPVVKLEKRLIAEYGMPSTHAMAATAI  
 AFTLLISTMDRYQYPFVLGLVMAVVFSTLVCLSRLYTGMHTVLDVLGGVL  
 30 ITALLIVLTPAWTFIDCLDSASPLFPVCVIVVPFFLCYNYPVSDYYSPT  
 RADTTTILAAGAGVTIGFWINHFFQLVSKPAESLPVIQNIPLTTYMLVL  
 GLTKFAVGIVLILLVRQLVQNLSLQVLYSWFKVVTRNKEARRRLEIEVPY



KFVTTYTSVGICATTFVPMLHRFLGLP (SEQ ID NO:43)

This amino acid sequence is predicted to contain 9 transmembrane domains by SMART and TmPred and 8 transmembrane domains by SOSUI. By contrast, when analyzed by use of the  
5 GENEID™ program, the following gene is identified as being overexpressed in colon tissue:

chr2\_2054 Nucleotide Sequence

ATGGCGGCCACTGCCATTGCCTTCACCCTCCTTATCTCTACTATGGACAG  
ATACCAGTATCCATTTGTGTTGGGACTGGTGATGGCCGTGGTGT TTTCCA  
10 CCTTGGTGTGTCTCAGCAGGCTCTACACTGGGATGCATACGGTCCTGGAT  
GTGCTGGGTGGCGTCCTGATCACCGCACTCCTCATCGTCCTCACCTACCC  
TGCCTGGACCTTCATCGACTGCCTGGACTCGGCCAGCCCCCTCTTCCCCG  
TGTGTGTCATAGTTGTGCCATTCTTCCTGTGTTACAATTACCCTGTTTCT  
GATTACTACAGCCCAACCCGGGCGGACACCACCACCATCTGGCTGCCGG  
15 GGCTGGAGTGACCATAGGATTCTGGATCAACCATTTCTTCCAGCTTGAT  
CCAAGCCCGCTGAATCTCTCCCTGTTATTGAGAACATCCCACCACTCACC  
ACCTACATGTTAGTTTTGGGTCTGACCAAATTTGCAGTGGGAATTGTGTT  
GATCCTCTTGGTTCGTCAGCTTGTAACAAAATCTCTCACTGCAAGTATTAT  
ACTCATGGTTCAAGGTGGTCACCAGGAACAAGGAGGCCAGGCGGAGACTG  
20 GAGATTGAAGTGCCCTTACAAGTTTGTTACCTACACATCTGTTGGCATCTG  
CGCTACAACCTTTGTGCCGATGCTTCACAGGTTTCTGGGATTACCCTGA  
(SEQ ID NO:44)

This gene encodes a protein having the following predicted structure:

25

chr2\_2054 Amino Acid Sequence

MAATAIAFTLLISTMDRYQYPFVLGLVMAVVFSTLVCLSRLYTGMHTVLD  
VLGGVLITALLIVLTYPAWTFIDCLDSASPLFPVCVIVVPFFLCYNYPVS  
DYYSPTRADTTTILAAGAGVTIGFWINHFFQLVSKPAESLPVIQNIPLT  
30 TYMLVLGLTKFAVGIVLILLVRQLVQNLSLQVLYSWFKVVTRNKEARRRL  
EIEVPYKFVTTYTSVGICATTFVPMLHRFLGLP\* (SEQ ID NO:45)

When this sequence is analyzed by SOSUI and TmPred it is predicted to possess 7 transmembrane domains. By contrast, analyses by SMART suggests that the protein has 5 transmembrane domains and a signal sequence. These analyses also indicate that the protein contains a PFAM domain indicating that the protein contains an acid phosphatase domain.

5

### AL531683

In a comparison of malignant colon samples with greater than 50% malignant cells in the sample against mixed normal tissues, fragment AL531683 was found to be upregulated 3.76-fold. The E-Northern analysis shown in Figure 14 demonstrates that the fragment is expressed in 100% of the tumors analyzed and poorly expressed in normal tissue.

10

### AL53168 Nucleotide Sequence

CGCCGGCGGTGCGTGTGGGAAGGCGTGGGGTGCGGACCCCGGCCCGACCTCNCCGTCCCGCC  
CGCCGCCTTCTGCGTCGCGGGNGCGGGCCGGCGGGGTCTCTGACGCGGCAGACAGNCCCTC  
15 GCTGTGCGCTCCAGTGGTTGTGCACTTGCGGGCGGCCCCCTCCGCGGCGGTGGGGGTGCCG  
TCCCGCCGGCCCGTCTGCTGCTGCCCTCTCNNGGGGGGTTTGCGCGAGCGTCGGCTCCGCCTGG  
GCCCTTGCGGTGCTCCTGGAGCGCTCCGGGTTGTCCCTCAGGTGCCCGAGGCCGAACGGTGG  
TGTGTGCTTCCCGCCCCCGGCGCCCCCTCCTCCGGTTCGCCCGCGGTGTCCGCGCGTGGGT  
CCTGAGGGAGCTCGTCGGTGTGGGGTTCGAGGCGGTTTGAGTGAGACGAGACGAGAC (SEQ  
20 ID NO: 46)

20

### AI202201

In a comparison of malignant colon samples with greater than 50% malignant cells in the sample against mixed normal tissues, fragment AI202201 was upregulated 3.18-fold. E-Northern analysis shown in Figure 15 demonstrates that the fragment is expressed in 77% of the tumors and poorly expressed in normal tissue.

25

### AI202201 Nucleotide Sequence

ACCCTATAGCTCCTTACGCTGGGAAAGCTGGTTTTTTAAAAAATAATAATAAAA  
30 TATTTAATCTTATTAAGTGTTCAATTTAAAATGCGTAATGCTTTGGAAATAATGGGTAACAGA  
TAGCGAGAGGATATGTTTATAAAGTGAGCATGTTGGTCCCATTTATAAATATATGTATGATT  
TATAAGCTTTTTTAAACAAAGCTCAAATTGTTGGTATTTTTCTAAAATGTGCACAGCTGTA

30

TTTTACATGAAGGCTCTTTCTAATGGGTTGTTATACTGTACTCAACATTTTGGACAGCACAT  
 GAAGTCTGCCAATGTACTTAATAAAACATGACTTTGTTTATTTAAAGTTTCTTGCTGTGAAA  
 AAGAACTCCCTACCTGTGAGTTCCTTTATTTATAATTCTTGAAACCAAATGTATAATGTAC  
 AGTTTTCACAACTGTATCTGCTCTAATA (SEQ ID NO:47)

5

#### AL389942

In a comparison of malignant colon samples with greater than 50% malignant cells in the sample against mixed normal tissues, fragment AL389942 was upregulated 3.83-fold. E-Northern analysis shown in Figure 16 demonstrates that the fragment is expressed in 55% of the tumors and poorly expressed in normal tissue.

10

#### AL389942 Nucleotide Sequence

GAAGCTCCAAATGCTCTGGGTTTCAGCTCCTCTGTGCTGTGGACNCTGACTTTGGCTCAGAA  
 CTCCGATTTAGTACAAAAGGCTCATTTTTATTTTCAGGGGCACTCTTCCTAAAGCAAACCTAA  
 TAAATGAAATATGGAATTCACAGATACACACACACATTAAAAAATTAACCTAGTGTATCTGT  
 GAGGAGTAGGCAGAAATTCNCTGTATAAAAGAATGCTTCATTTCATAGAGAATTTGTGTAA  
 GATTCCATTAGATAGTACATTTCTCAAAGATTTTGTAGGTTGTATTTGCTTTACCAAACCTT  
 GGTTTATGTAAGTGGAAAAAGCATGTTGCAAAATAACTTGGTGTCTATGATTCAGTTTATGT  
 AAAATAATAAATGTATGTAGGAATACGTGTGTTGAAAGATGTACATCAATTTGCTAACAATG  
 GTTATCTCTGACGTGGTGGGATTTGAGATGTGTTTTCTTTTGGTTGTATTTTCTCTATT  
 GTTTGACTTA (SEQ ID NO:48)

20

### EXAMPLE 5

#### Identification Of Gene Upregulated In Colon Cancer

Using the GeneLogic database and the methods described generally in Example 2, the following additional DNA sequences were identified as being overexpressed in colon tumor tissue:

25

30

DNA fragment NM\_021246 is 5-fold upregulated as shown by hybridization in the malignant colon when compared with mixed normal samples, greater than 3-fold upregulated compared with normal kidney, liver and lung, and greater than 2-fold upregulated in all other tissues.

## NM\_021246 Nucleotide Sequence

AACCGAATGCGGTGCTACAACCTGTGGTGGAAAGCCCCAGCAGTTCTTGCAAAGAGGCCGTGAC  
CACCTGTGGCGAGGGCAGACCCCAGCCAGGCCTGGAACAGATCAAGCTACCTGGAAACCCCC  
CAGTGACCTTGATTACCAACATCCAGCCTGCGTCGCAGCCCATCATTGCAATCAAGTGGAG  
5 ACAGAGTCGGTGGGAGACGTGACTTATCCAGCCCACAGGGACTGCTACCTGGGAGACCTGTG  
CAACAGCGCCGTGGCAAGCCATGTGGCCCCTGCAGGCATTTTGGCTGCAGCAGCTACCGCCC  
TGACCTGTCTCTTGCCAGGACTGTGGAGCGGATAGGGGGAGTAGGAGTAGAGAAGGGAACAA  
GGGAGCAAGGGAACAAGGGACATCTGAACATCT (SEQ ID NO:56)

- 10 The E-nothern results in Figure 17 indicate that this fragment is upregulated in colon and rectal malignancies. Accordingly, this gene can be targeted for the treatment of colon or rectal cancer. A search of commercial databases reveals that NM\_021246 is apparently part the Ly6G6D gene set forth below:

## 15 Ly6G6D mRNA Sequence

cccatggcagtcttattcctcctcctgttcctatgtggaactccccaggc  
tgcagacaacatgcaggccatctatgtggccttgggggagggcagtagagc  
tgccatgtccctcaccacctactctacatggggacgaacacctgtcatgg  
ttctgcagccctgcagcaggctccttcaccaccctggtagcccaagtcca  
20 agtggggcaggccagccccagaccctggaaaaccaggaaggggaatccaggc  
tcagactgctggggaactattctttgtggttgagggatccaaagaggaa  
gatgccgggcggtactggtgcgctgtgctaggtcagcaccacaactacca  
gaactggagggtgtacgacgtcttggtgctcaaaggatcccagttatctg  
caagggtgcagatggatccccctgcaatgtcctcctgtgctctgtgggtc  
25 cccagcagacgcatggactctgtgacctggcaggaaggggaaggggtccggt  
gaggggcccgtgttcagtccttctggggcagtgaggctgccttctctgg  
tgtgtcctggggaggggctttctgagcccaggagccgaagaccaagaatc  
atccgctgcctcatgactcacaacaaaggggtcagctttagcctggcagc  
ctccatcgatgcttctcctgccctctgtgccccttccacgggctgggaca  
30 tgccttggattctgatgctgctgctcacaatgggcccaggaggtgtcatc  
ctggccctcagcatcgtgctctggaggcagaggggtccgtggggctccagg  
cagaggaaaccgaatgcggtgctacaactgtggtggaagccccagcagtt

cttgcaaagagggccgtgaccacctgtggcgagggcagaccccagccaggc  
 ctggaacagatcaagctacctggaaacccccagtgaccttgattcacca  
 acatccagcctgcgctcgagcccatcattgcaatcaagtggagacagagt  
 cgggtgggagacgtgacttatccagcccacagggactgctacctgggagac  
 5 ctgtgcaacagcgccgtggcaagccatgtggcccctgcaggcattttggc  
 tgcagcagctaccgccctgacctgtctcttgccaggactgtggagcggat  
 agggggagtaggagtagagaaggggaacaagggagcaaggggaacaaggac  
 atctgaacatctaattgtgagaagagaaacatccttctgtgagtcattaaa  
 atctatgaaccactct (SEQ ID NO:57)

10

The amino acid sequence for Ly6G6D is set forth below:

#### Ly6G6D Amino Acid Sequence

MAVLFLLLFLCGTPQAADNMQAIYVALGEAVELPCPSPTLHGDEHLSWF  
 15 CSPAAGSFTTLVAQVQVGRPA PDGKPGRESRLRL LGNYSLWLEGSKEED  
 AGRYWCAVLGQHHNYQNWRVYDVLVLKGSQLSARAADGSPCNVLLCSVVP  
 SRRMDSVTWQEGKGPVRGRVQSFWGSEAALLLVCPGEGLSEPRSRRPRII  
 RCLMTHNKGVSFSLAASIDASPALCAPSTGWDMPWILMLLLTMGQGVVIL  
 ALSIVLWRQRVRGAPGRGNRMRCYNCGGSPSSSCKEAVTTCGEGRPQPGL  
 20 EQIKLPGNPPVTLIHQHPACVAAHHCNQVETESVGDVTYPAHRDCYLGDL  
 CNSAVASAVAPAGILAAAATALTCLLPGLWSG (SEQ ID NO:58)

Analysis of the Ly6G6D protein sequence using the SMART program identified two  
 potential transmembrane domains and an Ig domain, suggesting that this protein is a cell  
 25 surface protein.

### EXAMPLE 6

#### Identification of Colon-Cancer Associated Gene AI821606

##### FLJ32334

30 Fragment AI821606 set forth below, also was shown to be upregulated in colon, pancreas and  
 rectal malignancies. This is supported by the E-Northern results in Figure 18.

## AI821606 Nucleotide Sequence

TTCTCGGAGGGGCGTGGTGAGTCTCCAGTATGTTTCGGCCCAGCGCTCTTCGCACCCTTCT  
GGACCAAAGCGCCAAGGACTGCAGCCAGGAGAGAGGGGGCTCACCTCTTATCCTCGGCGACC  
CACTGCACAAGCAGGCCGCTCTCCAGACTTAAAATGTATCACCCTAACCTGTGAGGGGGA  
5 CCCAATCTGGACTCCTTCCCCGCCTTGGGACATCGCAGGCCGGGAAGCAGTGCCCGCCAGGC  
CTGGGCCAGGAGAGCTCCAGGAAGGGCACTGAGCGCTGCTGGCGCGAGGCCTCGGACATCCG  
CAGGCACCAGGGAAAGTCTCCTGGGGCGATCTGTAAAT (SEQ ID NO:51)

10 A database search revealed that AI821606 is in the 3'UTR of predicted genes corresponding  
to both strands of a chromosome. Based thereon, this fragment could be part of the  
following genes:

## ENST00000267803 Nucleotide Sequence

gcttccagcggacggcagcgcgagcattgccccccctgcaccacctca  
15 ccaagATGGCTACTTTGGGACACACATTCCCCTTCTATGCTGGCCCCAAG  
CCAACCTTCCCGATGGACACCACTTTGGCCAGCATCATCATGATCTTTCT  
GACTGCACTGGCCACGTTTCATCGTCATCCTGCCTGGCATTTCGGGGAAAGA  
CGAGGCTGTTCTGGCTGCTTCGGGTGGTGACCAGCTTATTCATCGGGGCT  
GCAATCCTGGGGACCCCCGTGCAGCAGCTGAATGAGACCATCAATTACAA  
20 CGAGGAGTTCACCTGGCGCCTGGGTGAGAACTATGCTGAGGAGTATGCAA  
AGGCTCTGGAGAAGGGGCTGCCAGACCCTGTGTTGTACCTAGCTGAGAAG  
TTCCTCCAAGAAGCCCATGTGGCCTATACCGCCAGTACCGCCTGGCGGG  
ACACTACACCTCAGCCATGCTATGGGTGGCATTCTCTGCTGGCTGCTGG  
CCAATGTGATGCTCTCCATGCCTGTGCTGGTATATGGTGGCTACATGCTA  
25 TTGGCCACGGGCATCTTCCAGCTGTTGGCTCTGCTCTTCTTCTCCATGGC  
CACATCACTCACCTCACCTGTCCCCTGCACCTGGGCGCTTCTGTGCTGC  
ATACTCACCATGGGCCTGCCTTCTGGATCACATTGACCACAGGACTGCTG  
TGTGTGCTGCTGGGCCTGGCTATGGCGGTGGCCCACAGGATGCAGCCTCA  
CAGGCTGAAGGCTTTCTTCAACCAGAGTGTGGATGAAGACCCCATGCTGG  
30 AGTGGAGTCTTGAGGAAGGTGGACTCCTGAGCCCCCGCTACCGGTCCATG  
GCTGACAGTCCCAAGTCCCAGGACATTCCCCTGTCAGAGGCTTCCTCCAC  
CAAGGCATACTGTAAGGAGGCACACCCCAAAGATCCTGATTGTGCTTTAt

aacattcctccccgtggaggccacctggacttccagtctggctccaaacc  
tcattggcgccccataaaaccagcagaactgccctcaggggtggctgttac  
cagacacccagcaccaatctacagacggagtagaaaaaggaggctctata  
tactgatgttaaaaaacaaaacaaaaagccctaaggggactgaaga  
5 gatgctgggcctgtccataaagcctgttgccatgataaggccaagcaggg  
gctagcttatctgcacagcaaccagcctttccgtgctgccttgcctctt  
caagatgctattcactgaaacctaacttcacccccataacaccagcaggg  
tgggggttacatatgattctcctatggtttccctctcatccctcggcacct  
cttggttttcccttttccctgggttcccttttggttcttccctttacttctccag  
10 cttgtgtggccttttggtacaatgaaagacagcactggaaaggaggggaa  
accaaacttctcatcctaggtctaacattaaccaactatgccacattctc  
tttgagcttcagttcccaaatttgctacataagattgcaagacttgccaa  
gaatcttgggatttatctttctatgccttgctgacacctaccttggccct  
caaacaccacctcacaagaagccaggtgggaagttagggaatcaactcca  
15 aaacgctattccttcccaccccactcagctgggctagctgagtggcatcc  
aggacgggggagtggggtgacctgcctcatcactgccacctaacgtcccc  
tgggggtggttcagaaagatgctagctctggtaggggtccctccggcctcac  
tagagggcgccccctattactctggagtcgacgcagagaatcaggtttcac  
agcactgcggagagtgtagctaggtgtctccagcccagcgaagctcatga  
20 ggacgtgcgaccccggcgcggagaagccatgaaaattaatgggaaaaaca  
gtttttaaaaaacaaaagaaaaaaagggtttatttacagatcgccccagga  
gactttccctgggtgcctgcggatgtccgaggcctcgcgccagcagcgctc  
agtgccttccctggagctctcctggcccaggcctggcgggcactgcttcc  
cggcctgcgatgtcccaaggcggggaaggagtcagattgggtccccctc  
25 acaggttagtggtgatacattttaagtctgggagagcggcctgcttgtgc  
agtgggtcgccgaggataagaggtgagccccctctctcctggctgcagtc  
cttggcgctttgggtccagaagggtgcgaagagcgctgggcccgaacatact  
ggagactcaccacggccccctccgaggaagaggcacaggacgcctgtggcg  
gtggggatcgaaagaaaggagggcatgtggagtcagggctatgttgccca  
30 ggctggtctcgaactctggcctcaaacgaccttctgcctcgacctcca  
aagtgctgggattacaggcgtgatgcccgggccttcttccatcttttggga  
gcctacccttgtgttacctcccgccacacacctctaactctgaattacat

gaaacacggcaagacaccaaacccttctgagccccccacttttcatctgt  
 aaaatggtcataacagtgcctgtttctgcaactattgagagggggcaa  
 agggtaatagatgtgaattcattctgtaaactgg (SEQ ID NO:52)

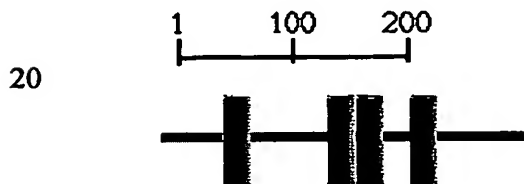
5 The predicted coding sequence for ENST00000267803 is set forth below:

#### ENST00000267803 Amino Acid Sequence

MATLGHTFPFYAGPKPTFPMDTTLASIIMIFLTALATFIVILPGIRGKTR  
 LFWLLRVVTSLFIGAAILGTPVQQLNETINYNEEFTWRLGENYAEYAKA  
 10 LEKGLPDPVLYLAEKFTPRSPCGLYRQYRLAGHYTSAMLWVAFLCWLLAN  
 VMLSMPVLVYGGYMLLATGIFQLLALLFFSMATSLTSPCPLHLGASVLHT  
 HHGPAFWITLTTGLLCVLLGLAMAVAHMQPHRLKAFFNQSVDEDPMLEW  
 SPEEGLLSPRYRSMADSPKSQDIPLSEASSTKAYCKEAHPKDPDCAL  
 (SEQ ID NO:53)

15

SMART analysis predicted that the protein contains several transmembrane domains (rectangles) and a signal sequence, as depicted schematically below:



Based on a sequence contained on the opposite strand of the chromosome, the following gene  
 25 sequence is predicted:

#### chr15.41.013.a Nucleotide Sequence

ATGACCCTGTGGAACGGCGTACTGCCTTTTTACCCCCAGCCCCGGCATGC  
 CGCAGGCTTCAGCGTTCCACTGCTCATCGTTATTCTAGTGTTTTTGGCTC  
 30 TAGCAGCAAGCTTCCTGCTCATCTTGCCGGGGATCCGTGGCCACTCGCGC  
 TGGTTTTGGTTGGTGAGAGTTCTTCTCAGTCTGTTCATAGGCGCAGAAAT  
 TGTGGCTGTGCACTTCAGTGCAGAATGGTTCGTGGGTACAGTGAACACCA



ACACATCCTACAAAGCCTTCAGCGCAGCGCGGTTACAGCCCGTGTCCGT  
 CTGCTCGTGGGCCTGGAGGGCATTAAATATTACACTCACAGGGACCCCAGT  
 GCATCAGCTGAACGAGACCATTGACTACAACGAGCAGTTCACCTGGCGTC  
 TGAAAGAGAATTACGCCGCGGAGTACGCGAACGCACTGGAGAAGGGGCTG  
 5 CCGGACCCAGTGCTCTACCTGGCGGAGAAGTTCACACCGAGTAGCCCTTG  
 CGGCCTGTACCACCAGTACCACCTGGCGGGACACTACGCCTCGGCCACGC  
 TATGGGTGGCGTTCTGCTTCTGGCTCCTCTCCAACGTGCTGCTCTCCACG  
 CCGGCCCCGCTCTACGGAGGCCTGGCACTGCTGACCACCGGAGCCTTCGC  
 GCTCTTCGGGGTCTTCGCCTTGGCCTCCATCTCTAGCGTGCCGCTCTGCC  
 10 CGCTCCGCCTAGGCTCCTCCGCGCTCACCCTCAGTACGGCGCCGCCTTC  
 TGGGTACGCTGGCAACCGGTGAGGACCGAGAGAATGGGCCCCGGGGGCT  
 AAGGGTGGAGACAGGATTACACCGGGCGTCCTGTGCCTCTTCCTCGGAG  
 GGGCCGTGGCCGGGAAGCAGTGCCCGCCAGGCCTGGGCCAGGAGAGCTCC  
 AGGAAGGGCACTGAGCGCTGCTGGCGCGAGGCCTCGGACATCCGCAGGCA  
 15 CCAGGGAAAGTCTCCTGGGGCGATCTGTAAA (SEQ ID NO:54)

This sequence is predicted to encode the following protein:

#### chr15.41.013.a Amino Acid Sequence

20 MTLWNGVLPFYPQPRHAAGFSVPLLIVILVFLALAASFLLILPGIRGHSR  
 WFWLVRVLLSLFIGAEIVAVHFSAEWFVGTVNTNTSYKAFFSAARVTARVR  
 LLVGLEGINITLTGTPVHQLNETIDYNEQFTWRLKENYAAEYANALEKGL  
 PDPVLYLAEKFTPSSPCGLYHQYHLAGHYASATLWVAFCFWLLSNVLLST  
 PAPLYGGLALLTTGAFALFGVFALASISSVPLCPLRLGSSALTTQYGAAF  
 25 WVTLATGEDRENGPRGLRVETGFTPGVLCLFLGGAVAGKQCPPGLGQESS  
 RKGTERCWREASDIRRHQKSPGAICK (SEQ ID NO:55)

SMART analysis identified three transmembrane domains (rectangles) and a signal sequence.

The predicted structure of the protein is depicted schematically below:

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